The Dissertation Committee for Lina Fernanda González Martínez Certifies that this is the approved version of the following Dissertation:

LONG-TERM EFFECTS OF SOCIAL STRESS EXPOSURE DURING ADOLESCENCE IN IMPULSIVITY: TOWARD A NEW MODEL OF AGGRESSION

Committee:

Yvon Delville, Supervisor

Hongjoo, Joanne Lee

Juan Dominguez

Hitoshi Morikawa

LONG-TERM EFFECTS OF SOCIAL STRESS EXPOSURE DURING ADOLESCENCE IN IMPULSIVITY: TOWARD A NEW MODEL OF AGGRESSION.

by

Lina Fernanda González Martínez

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

May 2019

Dedication

A mis papás y mis hermanas, quienes me han apoyado, creído en mí, entendido mi ausencia, consolado mis tristezas, compartido mis alegrías, y disipado mis miedos. Sin ellos esta tesis no sería posible.

(For my parents and sisters, whom have supported me, believed in me, understood my absence, comforted my sadness, shared my happiness, and eased my fears. Without them this dissertation would not have been possible).

Acknowledgements

I would like to gratefully acknowledge the guidance, support and encouragement of my doctoral advisor, Dr. Delville, and the members of my committee during my time at UT-Austin. I am profoundly grateful for having an incredible mentor, who has been an active and important part, of the process that helped me become a better person and a better scientist. Thank you Yvon for believing in me, always supporting me, and help me thrive as a Latina woman in science.

My gratitude extends to my lab-mate Kevin and all the undergrad students, for their enthusiasm and willingness to help and provide their expertise while running the experiments, and to Sarah and all the staff at the Animal Resources Center that took incredible care of my hamsters.

I am incredible grateful to my friends at UT Dr. Roberto Cofresí, Sandie Keerstock, Mayra Chantal Ramirez, and Dr. Alisa Baron for many hours of mutual support, complaining, insightful discussions, and non-science related talks that made this process more enjoyable. To my friends from Colombia who gave me their support in the distance, and didn't stop being present even when I was absent.

I owe my deepest gratitude to Patrick Drew for supporting me through the toughest times, always believing in me, and loving me unconditionally.

This dissertation would not have been possible without funding from The University of Texas at Austin, and the Colombian Department of Science, Technology and Innovation (COLCIENCIAS) through the scholarship program No 646.

Abstract

Long-term effects of social stress exposure during adolescence in impulsivity: toward a new model of aggression

Lina Fernanda González Martínez, PhD.

The University of Texas at Austin, 2019

Supervisor: Yvon Delville

Adolescent male hamsters exposed to chronic social stress become themselves aggressive adults, evidenced by increased frequency of attacks and shorter latencies to attack opponents. Perhaps, this enhanced aggression is associated with a lack of impulse control, in particular with the ability to inhibit responses (i.e. action inhibition) and wait to respond (i.e. waiting impulsivity). Male golden hamsters were exposed daily to aggressive adults from postnatal day 28 to 42. Later, the animals were trained in conditioning chambers and tested in a Go-NoGo task to evaluate action inhibition. Overall, previously stressed hamsters were less likely to inhibit a conditioned lever pressing response during NoGo trials. These results show that animals exposed to social stress in early adolescence, have a decrease ability to withhold responses, which could possible explain why as adults, they have higher frequency of attacks. To test waiting impulsivity, animals learned to respond to a main house-light by nose-poking in any of two, adjacent illuminated ports in a modified version of a 5-choice-serial-reaction-time task (5-CSRTT). During testing, random and varying delays were introduced between the main house-light presentation and illumination of the ports, and premature nose-poking responses, (i.e. responses before the

ports were illuminated) were considered an indicator of waiting impulsivity. As delays grew longer, animals performed more premature responses. However, previously stressed animals were 25% less likely to perform such actions by the longest delay. These studies show that early stress exposure enhanced the capacity to wait to perform a response, which is unrelated to aggression. Aspects of perseverance were tested in additional studies. In summary, chronic social stress exposure in early adolescence causes a variety of behavioral changes including enhanced aggression, decreased action inhibition and improved waiting impulsivity. This ambiguous relation between aggressive and impulsive behaviors suggests that perhaps there are multiple types of impulsive-aggression profiles related to different brain mechanisms. Thus, it is proposed that the concept of aggression should be reconsidered as a multidimensional construct mediating aspects of personality.

Table of Contents

List of Tables

List of Figures

CHAPTER 1: INTRODUCTION

Adolescence is a developmental period characterized by physical, cognitive and neuroendocrine changes. Some of these changes include sexual maturation through the development of the Hypothalamus-Pituitary-Gonadal axis (referred as puberty), brain development, and changes in social behavior, risk-taking, and impulsivity (Kestenberg, 1968; Larson, & Richards, 1991; Giedd, Blumenthal, Jeffries, Castellanos, Liu, Zijdenbos, Paus, Evans, & Rapoport, 1999; Cohn, Macfarlane, Yanez, & Imai, 1995; Green, Fry, & Myerson, 1994). All these changes make adolescence a vulnerable period for psychopathology, in fact during adolescence there is an increase in psychopathological symptoms, studied as prevalence and frequency of some disorders such as depression and anxiety, irritability and substance use (Mendle, 2014). However, adolescence is also a sensitive period for increased risk of subsequent adult psychopathology. In particular, some studies have shown that exposure to stress during adolescence is a risk factor for disorders like anxiety, depression, post-traumatic stress disorder (PTSD), and substance use during adulthood (Gladstone, Parker, & Malhi, 2006; Carlisle & Rofes, 2007; Silverman, Reinherz, & Giaconia, 1996).

Hypothalamic-Pituitary-Adrenal axis (HPA)

The Hypothalamic-Pituitary-Adrenal axis (HPA axis), the major stress response system, also undergoes maturation during adolescence. The HPA axis activates in response to stress, which is generally defined as a state of threatened homeostasis (physical or perceived threat to homeostasis), during which an adaptive compensatory specific response of the organism is activated to sustain homeostasis (Pacák, & Palkovits, 2001). The first element in the stress response is the activity of neurons in the medial parvocellular portion

of the paraventricular nucleus of the hypothalamus (PVN). These parvocellular neurons project to the hypothalamic median eminence and produce corticotropin releasing hormone (CRH) (Delville, Stires, & Ferris, 1992). In response to CRH, the anterior pituitary gland synthetizes and releases adrenocorticotropic hormone (ACTH) in the blood circulation. Lastly, in response to ACTH, cells in the adrenal cortex secrete the glucocorticoid hormones cortisol and/or corticosterone (CORT) into the systemic circulation affecting cells throughout the body. Once in the systemic circulation CORT produces negative feedback in the anterior pituitary and CRH neurons in the PVN (Selye, 1950; Chrousos, 1995).

The principal glucocorticoid in humans, most fish, primates and other mammals is cortisol, while corticosterone is found in birds, reptiles, amphibians, and some rodents like rats (Table 1). Glucocorticoid hormones are synthesized from a subset of adrenocortical cells in response to ACTH by the steroidogenic processing of cholesterol. These cells located in the zona fasciculate of the adrenal cortex respond to the binding of ACTH to its specific G-protein-coupled receptor the melanocortin type-2 receptor (MCR2). Following the binding of ACTH, MCR2 receptor undergoes conformational changes leading to the activation of adenylyl cyclase and subsequent increase in intracellular levels of cyclic adenosine mono phosphate (cAMP). Intracellular cAMP in turns activates protein kinase a (PKA) nuclear transcription factors, such as cAMP response element (CRE) and binding protein (CREB). Activation of these transcription factors induce the synthesis of glucocorticoids (Chung, Son, & Kim, 2011; Spiga & Lightman, 2015).

Species	Glucocorticoid	Reference
Fish		
Lamprey	11-deoxycortisol	Close, Yun, McCormick, Wildbill, & Li, 2010
Sharks/Ray	1α-hydroxycorticosterone +Corticosterone	Rasmussen & Crow, 1993 Truscott & Idler, 1972
Teleost and bony fish	Cortisol	Barton, Rahn, Feist, Bollig, Schreck, 1998; Mommsen, Vijayan, & Moon, 1999
Lungfish	Cortisol+Corticosterone+11-deoxyxcortisol	Idler, Sangalang, & Truscott, 1972
Amphibians		
Frogs	Corticosterone	Licht, McCreery, Barnes, & Pang, 1983; Leboulenger, Delarue, Tonon, Jegou, Leroux, & Vaudry, 1979
Newt	Corticosterone	Zerani & Gobbetti, 1993
Reptiles		
Alligator/Crocrodiles	Corticosterone	Guillette, Woodward, Crain, Masson, Palmer, Cox, You-Xiang, & Orlando, 1997; Lance & Lauren, 1984
Tortoise/Turtle	Corticosterone	Schramm, Casares, & Lance, 1999; Valente, Velarde, Parga, Marco, Lavin, Alegre, & Cuenca, 2011
Lizard/Snakes/Tuatara	Corticosterone	Tokarz, McMann, Seitz, & John-Alder, 1998 Tam, Phillips, & Lofts, 1972; Tyrrell & Cree, 1994
Birds		
All birds	Corticosterone	Siegel, 1980.; Cockrem, Barrett, Candy, & Potter, 2009; Wada, Hahn, & Breuner, 2007
Mammals		
Monotreme	Corticosterone	
Marsupials	Cortisol	Oddie, Blaine, Bradshaw, Coghlan, Denton, Nelson, & Scoggings, 1976
Koala	Corticosterone	
Carnivores (cats, dogs, ferrets)	Cortisol	Wildt, Phillips, Simmons, Chakraborty, Brown, Howard, Teare, & Bush, 1988; Reul, Rothuizen, & de Kloet, 1991; Accorsi, Carloni, Valsecchi, Viggiani, Gamberoni, Tamanini, & Seren, 2008
Seals	Cortisol+Corticosterone	Sangalang & Freeman, 1976
Cetaceas (Dolphin, whales)	Cortisol, Cortisol+Corticoster	St. Aubin, Ridgway, Wells, & Rhinehart, 1996
Bats	Cortisol	Gustafson, & Belt, 1981
Horse	Cortisol+Corticosterone	Zolovick, Upson, & Eleftheriou, 1966
Pig	Cortisol	Kattesh, Charles, Baumbach, & Gillespie, 1990
Elephants	Cortisol	Brown, & White, 1979
Manatee	Cortisol	Tripp, Verstegen, Deutsch, Bonde, de Wit, Manire, Gaspard, & Harr, 201
Hamster	Cortisol+Corticosterone	Dalle & Delost, 1976
Guinea Pig	Cortisol	Widmaier & Kinz, 1993
Rat	Corticosterone	Barrett & Stockham, 1963
Mice	Corticosterone	Spackman, Riley, Monjan & Collector, 1978; Bronson, 1973
Squirrel	Cortisol+Corticosterone	Adams, Biglieri & Bern, 1965
Tree Shrews	Cortisol	Collin, Tsang, & Metzger, 1984
Lemur	Cortisol	Cavigelli, 1999
Marmoset	Cortisol	Saltzman, Schultz-Darken, Scheffler, Wegne, & Abbott, 1994
Rhesus macaques	Cortisol	Bercovitch, & Clarke, 1995
Apes (Gorillas, chimpanzee)	Cortisol	Robbins & Czekala, 1997 Muller & Wrangham, 2004
Humans	Cortisol	Costa, Benedetto, Fabris, Giraudi, Testori, Bertino, Marozio, Varvello, Arisio, Ariano, & Emanuel, 1996

Table 1**.** Glucocorticoid secreted by different species.

The biosynthesis of glucocorticoids is related to various sets of genes and proteins. First, steroidogenic acute regulatory protein (StAR) mediates the transfer of cholesterol to the mitochondrial membrane, where it is converted into pregnenolone by the side chain cleavage enzyme, P450scc, which is coded by CYP11A1 (cholesterol side chain cleavage monooxygenase). Pregnenolone is then converted to progesterone, then to 11-deoxycorticosterone and ultimately into corticosterone. In animals producing cortisol, pregnenolone is converted into 17α-hydroxypregnenolone by CYP17 (17α-hydroxylase), then to 17α-hydroxyprogresterone, 11-deoxycortisol, to finally be converted into cortisol. Thus, the presence of CYP17 mediates the cleavage of pregnenolone and progesterone, constituting an additional pathway to cortisol biosynthesis (Nussey & Whitehead, 2001).

The basic structural requirement for a steroid to possess glucocorticoid activity is to be a carbon 21 (C-21) compound with a -CO-CH2OH side-chain attached at C-17. In addition, there is an unsaturated bond between C-4 and C-5 and a keto group (-C=O) at C-3 of ring A, which is primarily responsible for the tight binding to corticosteroid receptors. The presence of a hydroxyl group at C-11 conveys glucocorticoid activity to corticosterone, and the addition of a hydroxyl group at C-17 yields to cortisol (Joels & de Kloet, 1994; Nussey & Whitehead, 2001).

Glucocorticoids affect cells throughout the body by binding to their receptors. In the brain, there are two types of glucocorticoid receptors, the corticosteroid receptor type I, which responds positively to low levels of glucocorticoids, and the corticosteroid receptor type II, which responds to basal and stress levels (Reul & de Kloet, 1985). Corticosteroid receptor type II are usually intracellular, and are attached to heat shock proteins (hsp). These hsp are displaced when cortisol/corticosterone diffuses across cell membrane and binds to these receptors in target cells. Subsequent phosphorylation of the

receptors facilitates translocation of the hormone-receptor complex into the nucleus. This hormone-receptor complex with other transcription factors, such as c-jun and c-fos, can stimulate or inhibit the expression of specific genes (Nussey & Whitehead, 2001; Joels & de Kloet, 1994).

Corticosteroid receptors type II are widespread in the brain. Elevated densities are found within the hippocampus, caudate-putamen, locus coeruleus, neocortex, core of nucleus accumbens, bed nucleus of the stria terminalis, central amygdaloid nucleus, dorsolateral septum, septohypothalamic nucleus, thalamus, and hypothalamus (Ahima & Harlan, 1990). Corticosteroid receptors type I are widespread as well, including the hippocampus, lateral septum, medial and central amygdala, layer II of neocortex, anterior hypothalamus, and cerebellum (Ahima, Krozowski, & Harlan, 1991; Joels & de Kloet, 1994).

The HPA axis response to stress is modulated by a direct feedback effect of glucocorticoids on the hypothalamus and pituitary gland (Kretz, Reichardt, Schutz, & Bock, 1999; Jones, Hillhouse, & Burden, 1977; Mahmoud, et al., 1984). Furthermore, mediation of the HPA axis comes from glucocorticoids' action on extra hypothalamic regulatory centers, such as the hippocampus and prefrontal cortex. The hippocampus contains the highest concentration of glucocorticoid receptors after the pituitary and hypothalamus (Rhees, Grosser, & Stevens, 1975; Gerlach, & McEwen, 1972; Alexis, Stylianopoulou, Kitraki, &. Sekeris, 1983) and constitutes the limbic structure with the highest uptake of labeled corticosterone (McEwen, Weiss, & Schwartz, 1969). Several studies have shown that hippocampal lesions increase glucocorticoids basal and peak levels, evidencing its participation in the negative feedback of the HPA axis through its projections to PVN (Magariños, Somoza, & De Nicola, 1987). In the same way, studies

evaluating lesions of the medial prefrontal cortex have shown increased peak levels of corticosterone (Diorio, Viau, & Meaney, 1993) supporting its role on the modulation of the stress response.

Glucocorticoid hormones resulting from the stress response are involved in the regulation of several bodily functions including glucose metabolism, and activation of anti-inflammatory and immunosuppressive responses (Munck, 1971; Hench, Kendall, Slocumb, & Polley, 1949; Bateman, Singh, Kral, & Solomon, 1989).

HPA AXIS AND ADOLESCENCE

As mentioned earlier, the activity of the HPA axis varies during development. In rodents, four major developmental stages can be identified: newborn (P1-P15), infancy (P15-P28), puberty/adolescence (P28-P69) and adulthood (P70). The criterion to differentiate these stages is mostly behavioral, as newborns are totally dependent of dams and are barely able to walk. Infants on the other hand, are very social but still rely on dams for protection and feeding. Juvenile animals have been weaned and are learning to be independent animals, while adults are totally independent. However, some species vary in the stage of development at birth, thus while some species are altricial and are very immature and helpless at birth, others are precocial and are very well developed and may survive with little parental care (Nelson, 2005). Thus, the timing and relative duration of infancy and newborn periods may vary between species.

Adolescence in male hamsters starts at postnatal day 28 (P28) and goes until P65-70. This period can be further subdivided in early adolescence characterized by low androgen production and play fighting behavior (P28-P40); mid adolescence (P40-P49) characterized by an activation of the androgen production, transition from play fighting to

aggression, and low sexual appetitive behavior; and late adolescence (P50-P69) characterized by full androgen production, adult aggression and sexual appetitive behaviors. After P70, all rodents are usually considered fully mature adults (Vomachka, & Greenwald, 1979; Wommack, Salinas, Melloni, & Delville, 2004).

A hyporesponsive stress period has been identified in newborns (P1–12). During this period, stimuli which normally elicit a significant elevation of corticosterone levels in adults, fail to do so in pups. Baseline plasma glucocorticoid levels are lower than normal and are only minimally increased by exposure to certain stressor, such as maternal separation (Guillet, Saffran, & Michaelson, 1980; Butte, Kakihana, Farnham, & Noble, 1973; Suchecki, Rosenfeld, & Levine, 1993).

During infancy, starting at P12 there is an increase in HPA axis activity. In general, hypothalamic CRH content, and pituitary ACTH content increase gradually with age (Walker, Perrin, Vale, & Rivier, 1986). In the same way, corticosteroid type II receptors are low at 3 days after birth and rise thereafter reaching adult levels at approximately the same time that corticosterone levels do (Meaney, Sapolsky, & McEwen, 1985).

During adolescence, there are differences between species in relation to the maturation of the HPA axis. At least three patterns can be identified (Table 2):

1. Stable. The first pattern consists of a dramatically increase in basal corticosterone levels that peaks before puberty. Afterwards corticosterone decreases and by early adolescence, basal levels are adult-like showing a stable basal corticosterone levels during adolescence. This pattern of HPA axis maturation is observed in Sprague-Dawley, Long Evans rats, white face capuchin monkeys, and rhesus macaque (Henning, 1978; Henning & Genovese, 1985; Walker, et al., 1986; Ordyan,

Galeeva, & Pivina, 2008; Meaney, et al., 1985; Jack, et al., 2014; Conley, Plant, Abbott, Moeller, & Stanley, 2011; Feng, et al., 2016).

In Sprague-Dawley rats the response to a single stress exposure during adolescence is characterized by a later peak response, and a delayed return to basal corticosterone and ACTH levels compared to adults. Immediately after exposure to stress, both adolescent and adult animals show an increase in basal corticosterone levels, nevertheless adult animals have its maximal corticosterone release faster than adolescents do. After peak, corticosterone levels in young animals remain elevated well beyond the time in which these levels decline in adult animals. Thus, corticosterone levels return to baseline faster in adults than in juveniles as the feedback system improves with enhanced production of corticosteroid type II receptors. (Vazquez & Akil, 1993; Romeo, Lee, Chhua, McPherson, & McEwen, 2004; Lui, et al., 2012; Doremus-Fitzwater, Varlinskaya, & Spear, 2009). Similar findings have been observed in Long-Evans rats, in which a later peak of corticosterone levels and a slower recovery is observed in adolescents compared to adults (Goldman, Winget, Hollingshead, & Levine, 1973; McCormick, Smith, & Mathews, 2008). Thus, the main difference between adolescent and adults with this pattern of HPA axis maturation is the speed of the negative feedback after a single stress exposure.

2. Increase. The second pattern of HPA axis maturation includes a progressive augmentation of basal corticosterone levels that peaks in middle adolescence, and stabilizes through late adolescence, showing an increase in basal corticosterone levels throughout the entire period of adolescence. This pattern is primarily observed in Wistar rats, Golden Hamsters, tree shrews, and eastern chimpanzee

(Pignatelli, Xiao, Gouveia, Ferreira, & Vinson, 2006; Caceres, et al., 2014; Wommack, et. al., 2004; van Kampen & Fuchs, 1998; Seraphin, Whitten, & Reynolds, 2008). Thus, in these species, baseline and peak post-stress levels increase gradually during adolescence (Cruz, DeLucia, & Planeta, 2005).

In hamsters, the HPA axis response to a single stress exposure increases during adolescence. Specifically, cortisol levels after a single session of restraint stress increase across adolescence, with levels at P45 higher than levels at P28, and levels at P70 higher than levels at P45 (Wommack, Salinas, & Delville, 2005). In the same way, exposure to chronic social stress from P28 to P42, show increased post-defeat cortisol levels during adolescence (Wommack & Delville, 2003; Wommack, et. al., 2004).

To my knowledge there are no studies comparing glucocorticoid levels in response to stress between adolescent and adult animals with a pattern 2-Increase of HPA axis development. Nevertheless, the studies available suggest that corticosterone levels right after 30 minutes of restraint stress are higher in adults compared to adolescent in hamsters and Wistar rats (Wommack, Salinas, & Delville, 2005; Bourke & Neigh, 2011). Additionally, the glucocorticoid levels observed after 30 minutes of restrain stress in adolescent (Cruz, Marin, Leão, & Planeta, 2012) and adult Wistar rats (Marin, Cruz, & Planeta, 2007) in two separate studies, suggest that adults have a faster recovery rate with higher levels of corticosterone 60 min after stress offset, and return to basal levels after 120 min (Marin, Cruz, & Planeta, 2007). On the other hand, it seems that adolescent Wistar rats, although still have levels higher than baseline 60 min after stress offset (Cruz, Marin, Leão, & Planeta, 2012), this level seems higher than adult's and suggest a slower recovery rate.

3. Decrease. The third pattern of HPA axis maturation includes a dramatically increase in basal corticosterone levels that peaks in infancy. Afterwards, basal levels decrease to a minimum in adulthood. This pattern is observed in basal and peak post-stress responses. Thus, the key feature in this pattern is a decrease in basal corticosterone levels during adolescence (especially early adolescence). This pattern is primarily observed in BALB/c, CD1 mice, vervet monkeys, guinea pigs, wild cavies, and zebra finches (Spinedi, Chisari, Pralong, & Gaillard, 1997; Schmidt, Enthoven, van der Mark, Levine, de Kloet, & Oitzl, 2003; Laudenslager, Jorgensen, & Fairbanks, 2012; Zipser, Schleking, Kaiser, & Sachser, 2014; Bölting & von Engelhardt, 2017).

   In CD1 mice, during the first week of age expression levels of CRH mRNA in the PVN are relatively high. However, CRH expression after two weeks significantly decreases compared to the earlier ages and remain at this level until the beginning of adolescence (Schmidt, et al., 2003).

   In BALB/c mice it has been observed that prepubertal and adult animals show similar corticosterone levels in response to stress, but prepubertal animals show higher peak levels right after the termination of the stress compared to adults, and similar recovery rates in both ages (Romeo, Kaplowitz, Ho, & Franco, 2013).

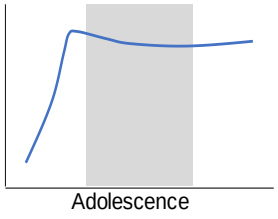
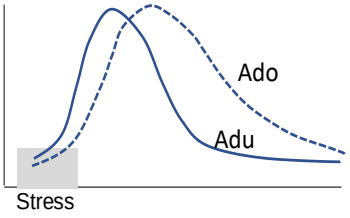
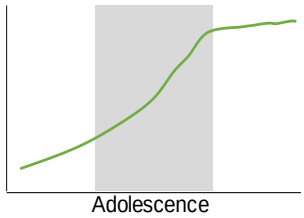
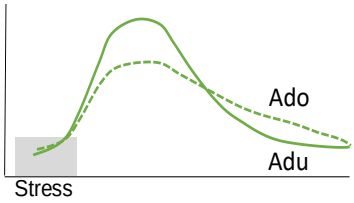
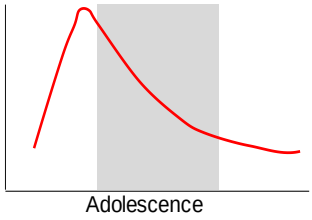
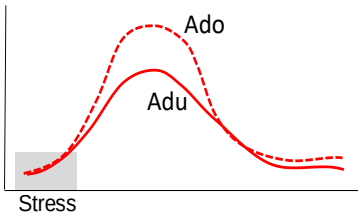
Basal Glucocorticoid levels	Glucocorticoid levels in response to stress	Potential effects of stress
1. Stable		
		More severe in early adolescence
Sprague-Dawley, Long Evans rats, white face capuchin monkeys, rhesus macaque, humans (?)		
2. Increase		
		Less severe in early adolescence. More severe in late adolescence/adulthood
Wistar rats, Golden Hamsters, tree shrews, eastern chimpanze, humans (?)		
3. Decrease		
		More severe in early adolescence
BALB/c, CD1 mice, vervet monkeys, guinea pigs, wild cavies, zebra finches		

Table 2. Developmental patterns of HPA axis maturation. In glucocorticoid levels in response to stress: solid lines represent adults (Adu), and broken lines represent adolescence (Ado).

HPA axis development in Golden Hamsters

In the present studies, Golden Hamsters (Mesocricetus auratus) are used. Hamsters' adrenal cortex is capable of secreting both cortisol and corticosterone. Both glucocorticoids are present in plasma in basal light and dark phase of the cycle, however, corticosterone is higher than cortisol at the beginning of the light phase (Ottenweller, Tapp, Burke, &

Natelson, 1985), while cortisol is higher during the dark phase of the cycle (Albers, Yogev, Todd, & Goldman, 1985). As mention before, in golden hamsters, basal plasma cortisol levels increase gradually during adolescence from postnatal day 28 to 70 (Wommack, et al., 2004), suggesting a type 2-Increase pattern of development. Additionally, the density of CRH fibers projecting from the PVN into the median eminence, increases significantly from early adolescence (P35) to adulthood (P70), suggesting that all elements of the HPA axis increase during adolescence in male golden hamsters (Wommack, Salinas, & Delville, 2005).

HPA axis development in humans

In humans there is evidence suggesting both a type 1-Stable and a type 2-Increase pattern of HPA axis development. Evidence supporting a type 1-Stable pattern has shown that cortisol levels remain stable across adolescence (Fadalti, et al., 1999; Knutsson, et al., 1997). One study, compared two groups of individuals including pre to early puberty (PEP), and mid to post-puberty (MPP). It was observed that MPP girls had significantly higher morning cortisol levels than MPP boys. However, no significant differences in cortisol levels between PEP and MPP were observed, suggesting a pattern 1-Stable of HPA axis development with constant basal corticosterone levels during adolescence (Netherton, Goodyer, Tamplin, & Herbert, 2004).

On the other hand, some evidence has shown that there is a gradual rise in cortisol during middle childhood, then a marked increase that begins at 13 years of age and continues through adolescence (Walker, et al., 1995) supporting the idea that cortisol increases with age during adolescence, and that humans have a type 2-Increase pattern of development. Some evidence has shown that as the HPA axis matures throughout

adolescence, so it does the response to stress (Stroud, et al., 2009; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). In particular, it has been observed in adolescence an increase in emotional reactivity, cardiovascular response and overall startle response (Silk, et al., 2009; Stroud, et al., 2009; Quevedo, Benning, Gunnar, & Dahl, 2009).

Gender differences have been found in relation to the HPA axis maturation that could account for the differences observed in humans. Some studies have found that girls show and increase in basal corticosterone through adolescence (Stroud, Papandonatos, Williamson, & Dahl, 2011; Legro, Lin, Demers, & Lloyd, 2003). Specifically, it has been observed that daytime cortisol levels were higher in post-menarche females compared to pre-menarche females, especially in the middle of the day (6h post-awakening) (Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2009). A separate study found a progressive but slow increase in girl's cortisol levels from 7.5 to 18.5 years (Apter, Pakarinen, Hammond, & Vihko, 1979); however, it is worth mentioning that in this study bone age was used instead of stage of development (Tanner stages).

On the other hand, in boys it has been observed a different patter. Specifically, it has been observed a small decrease in baseline cortisol across adolescence (Stroud, et al., 2011). Nevertheless, it has also been observed that boys' cortisol first displays a significant decrease until about 12 years of age, and then increases after 16.5 years (Apter, et al., 1979); however, it is worth mentioning that in this study bone age was used instead of stage of development (Tanner stage).

Significance of different types of HPA axis maturation

The differences between patterns of HPA axis maturation, suggest that the potential consequences of prolonged corticosteroid exposure due to stress exposure in adolescence could be different depending on the developmental pattern (Table 2). In the case of animals with type 1-Stable or type 3-Decrease, in which at adolescence animals have recently been exposed to peak developmental levels of glucocorticoids, it would be expected that stress exposure in this stage has more dramatic effects than in adulthood. On the other hand, stress exposure during adolescence in species with patter 2-Increase may have different effects, with stronger effects during adulthood and late adolescence than in early adolescence.

Some evidence has supported these differences between species and different patterns of HPA axis maturation. Sprague-Dawley rats exposed to acute restriction during adolescence spent less time in the light, had shorter latencies to escape the light, and longer times to return to the light in a light/dark box, compared to adults exposed to restriction, suggesting that restrain significantly altered the behavior of adolescents but not adults in this task (Slawecki, 2005). In the same way, Long-Evans rats exposed to nicotine for 15 days either during adolescence (P28–42) or adulthood (P85–99), showed less center time in an open field in adolescence compared to controls. On the hand, on a fear conditioning task, adolescent animals pre-treated with nicotine had superior acquisition but failed to extinguish the response. In contrast, animals pre-treated during adulthood did not have any significant difference with controls on either task (Smith, et al., 2006). Additionally, C57BL/6J mice isolated for 40 days after weaning showed an increase in ethanol intake compared to grouped-house animals. However, when animals were isolated for the same duration during adulthood (P60), subsequent ethanol intake was not altered relative to consumption in group-housed controls (Lopez, Doremus-Fitzwater, & Becker, 2011).

On the other hand, in animals with a type 2-Increase pattern it will be expected that stress exposure during adulthood has potentially more severe effects than during adolescence. In relation to this possibility, there has been a unique effect of stress during adolescence in aggression observed in animals with a type 2-Increase pattern of HPA axis maturation. Male adolescent golden hamsters exposed to social-defeat are more likely than controls to be aggressive toward a smaller, younger and submissive animal intruder. While these animals are less likely to attack a same age and weight intruder compared to controls (Delville, Melloni, & Ferris, 1998), these defeated animals in the presence of a smaller and younger animal as adults (P70) showed increased aggression (Wommack, et al., 2003). This same effect of increased aggression in adulthood has been also observed in Wistar Hans rats exposed to variable stress during adolescence. In particular, these rats show an increased in the amount of time spent keeping down the intruder compared with controls (Tzanoulinou, Riccio, de Boer & Sandi, 2014; Márquez, et al., 2013). Contrary to this, when adult male hamsters are exposed to social-defeat, the complete opposite is observed. These defeated adults display submissive behaviors and lack of aggression in the presence of any intruder, regardless of size and age (Potegal, Huhman, Moore, & Meyerhoff, 1993; Jasnow, Banks, Owens, & Huhman, 1999).

A clustered meta-analysis on stress-induced alcohol consumption, showed that male adult Wistar rats are more sensitive to stress-induced increases in alcohol drinking than most other rat strains, especially when exposed to either foot shock or forced swim stress within the context of a free-choice home cage drinking paradigm (Noori, Helinski & Spanagel, 2014). Furthermore, some evidence supports the role of glucocorticoids in the facilitation of alcohol consumption. Specifically, it has been observed that adrenalectomy causes decreased alcohol-drinking, whereas corticosterone administration increases

voluntary alcohol intake in Wistar rats (Fahlke, Engel, Eriksson, Hård, & Söderpalm, 1994; Fahlke, Hård, & Hansen, 1996). Taking into account that Wistar rats have a type 2-Increase pattern of HPA axis maturation, this evidence supports the idea that stress exposure at different developmental periods (i.e. adolescence or adulthood) in species with different patterns of HPA axis maturation will have specific and particular consequences, as observed in the case of stress-induced alcohol consumption.

Types of Stressors

Stress responses can be triggered by exposing the animals to different situations that will activate different compensatory mechanism associated with specific systems, and therefore can have different consequences. Stress procedures can be divided into physical and psychosocial. Some of the physical procedures include:

- Restraint and immobilization stress. In these procedures, animals' locomotion is restricted by being placed in a cylindrical tube with ventilation holes, or by gently wrapping of their upper and lower limbs (Pitman, Ottenweller, & Natelson, 1988). Some studies have found than animals do not habituate to repeated exposure to restraint/immobilization (Pitman, et al.,1988), while others have reported that animals do (Lachuer, Delton, Buda, & Tappaz, 1994). The duration and frequency of restraint or immobilization varies greatly across studies, as well as the moment in the light/dark cycle in which stress is presented. These differences could explain the variability in the adaptation response to the stress exposure found in some studies, raising questions about the reliability of the behavioral effects observed after restrain/immobilization exposure.

- Electric foot shock. In this procedure, animals are placed in a chamber with a metal grid floor, and after habituation to the chamber, they receive mild, brief foot shocks (Báez, Siriczman, & Volosin, 1996). The behavioral effects observed with electric foot shook are consistent across studies (Seligman, & Beagley, 1975; Jackson, Alexander, & Maier, 1980; Pynoos, Ritzmann, Steinberg, Goenjian, & Prisecaru, 1996); however, questions have been raised about the ecological validity of this method as a stress procedure. Some evidence has suggested a possible adaptation against repeated foot shock stress exposure. However, the characteristics of test procedure including stress intensity and measures of stress may possibly influence the results regarding the existence and non-existence of stress adaptation (Anjana Bali & Jaggi, 2015).
- Temperature variation stress. It consists on changing the body temperature, generally by immersion in cold water. Although behavioral effects have been observed in acute presentation, chronic exposure leads to habituation (Agrawal, Jaggi, & Singh, 2011).
- Noise. In this procedure loud noise (white noise or a bell) is presented above the home cages for different durations and frequency of presentations. Acute presentations of noise stressor generate increase concentrations in several hormones, however multiple exposures to noise generates habituation to the stressor (de Boer, Van Der Gugten, & Slangen, 1989; Armario, Castellanos, & Balasch, 1984).

Some of the psychosocial stress procedures include:

- Maternal separation and social isolation. Maternal separation involves separation of the litter from the dam, generally for more than 3 hours per day in the first 2

weeks after birth. Pups can be placed either individually or kept together in litters. Social isolation involved single housing the animals after weaning and for longer periods. This procedure can be used to prevent social interactions at different ages. Nevertheless, since social behavior and social interactions differ before and after weaning, it is important to distinguish between the effects elicited by each procedure (Nylander, & Roman, 2013). Many differences in the duration and frequency of these procedures, the fact that pups still do not regulate their body temperature when separated, and that while separated pups are deprived from food and maternal care, make difficult to compare results, especially with maternal separation (Lehmann, & Feldon, 2000).

- Predator odor. This procedure is based on a natural threat for animals, a predator odor, that generally triggers natural physiological and behavioral responses. Some of the behaviors observed after exposure to predator odor include freezing, avoidance, and risk assessment. Habituation to predator odor presentation has been observed in some studies however the amount of odor presented seems to be the determinant to trigger or not habituation (Takahashi, Nakashima, Hong, & Watanabe, 2005). Nevertheless, some studies have shown no short or long-term behavioral effects after chronic predator exposure (Chen, Shen, Liu, & Li, 2014), perhaps because it is natural for prey species to respond to predators and overcome those responses.
- Chronic unpredictable stress. This procedure consists of randomly presenting different stressors on a daily basis preventing the habituation observed in other stress models. Animals can be exposed to a various stressor, including: foot-shocks, restraint, changes in home-cage environment, increased noise, and restricted access

to food or water (Willner, Towell, Sampson, Sophokleous, & Muscat, 1987). Nevertheless, the nature of each stress is different, and so it is the subjacent neuroendocrine system related to each one; therefore, is hard to know which stress is related to the behavioral consequences observed. For example, while immersion in cold water or administration of foot-shock has an effect on sexual behavior in male rats, immobilization does not (Retana-Márquez, Bonilla-Jaime, Vázquez-Palacios, Martínez-García, & Velázquez-Moctezuma, 2003).

- Social instability. This procedure involves pair-housed animals that are removed from the colony room for one hour, after which they are returned to the colony but to a new cage in which they are paired with a new cage partner of the same age. It has been observed that adolescent male rats habituate to daily isolation, and show sensitized response to repeated pairing with a new cage partner, while adult rats show habituation to both isolation and to unfamiliar partners (Hodges, & McCormick, 2015; McCormick, 2010).
- Social stress (defeat). This model is based on the natural occupation and establishment of a territory by a resident male, and the fact that he will defend that territory against unfamiliar male intruders. For this, a resident-intruder paradigm is used. In this paradigm a male intruder is placed in the home cage of another resident male, allowing generally direct contact that leads to interaction, fighting and biting. These interactions lead to an animal winning the fights and establishing as dominant, and the one that loses the fights displaying submissive behaviors and establishing as the defeated animal (Koolhaas, Meerlo, de Boer, Strubbe, & Bohus, 1997; Bjorkqvist, 2001; Tidey & Miczek, 1996). Social subordination results in a variety of consequences such as: increased basal corticosterone levels (Blanchard,

et al., 1995), decrease testosterone (Huhman, Moore, Ferris, Mougey, & Meyerhoff, 1991; Wommack, et al., 2004), conditioned defeat (Potegal, et al., 1993), avoidance (Bastida, Puga, & Delville, 2009), and aggression (Wommack, Taravosh-Lahn, David, & Delville, 2003).

Taking into account the different forms of stress, it is important to distinguish that different types of stress, as well as the stage of development in which it is presented, will determine the short- and long-term consequences observed. Because the studies in this dissertation use social defeat in adolescence, for the remaining of this dissertation I will focus on social stress.

SHORT-TERM EFFECTS OF SOCIAL STRESS IN ADOLESCENCE

The short-term effects of social stress exposure in adolescence have been evaluated in a variety of tasks and have shown a wide range of effects (Table 3). The wide range of effects observed may be caused by the different species used, the time in development that the animals were exposed to stress, the different housing conditions (i.e. single-housed, pair-housed), and the differences in the protocol used for stressing the animals.

In particular, it has been observed an increase in measures considered for anxiety in the elevated plus maze, without changes in measures in the open field test. In particular, animals exposed to social stress during adolescence and tested shortly after the stress exposure have shown higher entries and percentage of time spent in the closed arms of an elevated plus maze compared to non-stressed controls. This has been observed in male c57BL/6J mice (Kovalenko, et al., 2014; Iñiguez, et al., 2014), and Long-Evans rats (McCormick, et al., 2008). However, no effect on the elevated plus maze was observed on Wistar rats that were single-housed and exposed to social defeat and restrain stress in early

adolescence (Bourke & Neigh, 2011). Additionally, when tested in an open field, no significant differences have been observed with controls in male c57BL/6J mice exposed to social-defeat in early adolescence (Kovalenko, et al., 2014), or Sprague-Dawley rats exposed to social stress in early, mid or late adolescence (Bingham, et al., 2011). In a separate study, in order to test the capacity of animals to control their levels of anxiety in different contexts, the authors tested Wistar rats for contextual fear against the need to drink water induced by water deprivation. In this case, rats exposed to social-defeat in mid-adolescence and tested in the water conflict test, consumed less water, spent less time drinking and showed higher latency to start drinking in comparison to control rats when tested in the defeated context. When animals were tested in an unfamiliar context or in the home cage, there were no differences in any measure between defeated and control animals. Thus, exposure to social-defeat in adolescence has short-term consequences that are specific to the defeat context (Vidal, Buwalda, & Koolhaas, 2011a).

In tests for depressive-like states such as the forced-swim test, contradictory results have been observed between increase and decrease immobility. It has been observed that male c57BL/6J mice spent more time in passive behavior compared to controls (Kovalenko, et al., 2014), but also showed shorter latencies to adopt a posture of immobility and spent more time immobile (Iñiguez, et al., 2014). On the other hand, adolescent Sprague-Dawley, Long-Evans and Wistar rats exposed to social stress do not differ from control rats in swimming or immobility behavior (Bingham, et al., 2011; Mathews, Wilton, Styles, & McCormick, 2008; Bourke & Neigh, 2011). Additionally, the effects of socials tress have also been evaluated in the sucrose-preference test. This test has been used as an indicator of anhedonia, a state characterized by inability to experience pleasure related to depression. In this case, adolescent male c57BL/6J mice defeated in

mid-adolescence, showed decrease preference for a sucrose solution compared to control animals (Iñiguez, et al., 2014). However, single-housed Wistar rats exposed to social defeat and restrain stress did not differ from control animals in this test (Bourke & Neigh, 2011).

Using the defensive burying test to evaluate animals' response to a threatening or noxious object, it has been observed an increase or decrease in the active burying behavior depending on the age of the animals. In particular, while Sprague-Dawley exposed to social-defeat in early adolescence showed increased defensive burying behavior, evidenced by an increased duration of burying and a decrease latency to begin compared to controls, exposure to social-defeat in late adolescence decreased defensive behavior duration compared to controls. On the other hand, exposure to social-defeat in mid-adolescence did not have any significant effect on defensive burying behavior (Bingham, et al., 2011).

When tested in an object recognition memory task, Long-Evans rats exposed to social instability during mid-adolescence, did not differ from control rats and spent more time exploring a novel than a familiar object, regardless if there was a novel object or if it was a known object in a new location (McCormick, et al., 2012).

Furthermore, animals exposed to social stress in adolescence showed social avoidance. This has been evidenced in male c57BL/6J mice, when tested for approaches and time spent near an empty cylinder or a cylinder containing an unfamiliar animal (Kovalenko, et al., 2014; Iñiguez, et al., 2014; Zhang, Yuan, Shao, & Wang, 2016). This has also been observed in defeated adolescent hamsters, which showed avoidance toward adult males (Bastida, et al., 2009), as well as non-receptive females (Bastida, et al., 2015) when tested in a Y-maze that allowed examination of the motivation of subjugated animals in each arm of the maze in the presence of a social or a non-social stimulus.

The short-effects of social stress exposure in adolescence have also been evaluated in the context of aggression. In this case, it has been observed that male golden hamsters exposed to social-defeat in early adolescence were more likely than controls to be aggressive toward a smaller, younger and submissive animal intruder. This was evidenced by shorter latencies to attack and increased number of bites. Previously stressed animals tested with a same age and weight animal, were less likely to attack the intruder than controls. This was evidenced by a smaller number of attacks and increased number of retreats. Thus, the exposure to social defeat during adolescence results in a context-dependent alteration of aggressive behavior, with increase aggression toward smaller and younger animals, and not evidence of aggression and a tendency to retreat when the intruder was the same age and weight (Delville, Melloni, & Ferris, 1998).

Short-term effects of social stress			
Species	**Task**	**Effect**	**Reference**
c57BL/6J	EPM	Higher entries and percentage of time in close arms	Kovalenko, et al., 2014; Iñiguez,et al., 2014
Long-Evans	EPM	Higher entries and percentage of time in close arms	McCormick, et al., 2008
Wistar	EPM	=	Bourke, & Neigh, 2011
c57BL/6J	Open field	=	Kovalenko, et. el, 2014
Sprague-Dawley	Open field	=	Bingham, et al., 2011
Wistar	Water conflict test	Consumed less water, less time drinking and higher latencies to star drinking when tested in defeat context. No effect when tested in unfamiliar context or home cage.	Vidal, et al., 2011a
c57BL/6J	Forced swim	More time in passive behaviors, shorter latencies to immobility, and more time immobile	Kovalenko, et al., 2014; Iñiguez, et. al., 2014
Sprague-Dawley	Forced swim	=	Bingham, et. al., 2011
Long-Evans	Forced swim	=	Mathews, et al., 2008
Wistar	Forced swim	=	Bourke, & Neigh, 2011
c57BL/6J	Sucrose preference	Decrease preference for sucrose solution	Iñiguez, et. al., 2014
Wistar	Sucrose preference	=	Bourke & Neigh, 2011
Sprague-Dawley	Deffensive burying	Increased duration burying and decrease latency to begin when exposed to stress in early adolescence. Opposite effect when stressed in late adolescence	Bingham, et. al., 2011
Long-Evans	Object-Recognition	=	McCormick, et al., 2012
c57BL/6J	Social approach-avoidance	Social avoidance	Kovalenko, et al., 2014; Iñiguez, et. al., 2014; Zhang, et al. 2016
Hamsters	Social approach-avoidance	Avoidance toward adult males and non-receptive females	Bastida, et al., 2009; Bastida, et al., 2015
Hamsters	Aggression	Shorter latenices to attack and increased number of bites toward a smaller, younger and submisive intruder. Opposite efect when intruder was same age and weight.	Delville, et al., 1998

Table 3. Short-term effects of social stress exposure in early adolescence. Only studies using males are including. EPM: Elevated-Plus-Maze. = no significant difference with control group.

LONG TERM EFFECTS OF SOCIAL STRESS IN ADOLESCENCE

Long-term effects of social stress exposure in adolescence have been evaluated in a variety of tasks and have shown a wide range of effects (Table 4). In general, it has been

observed increased locomotion, and generalized anxiety and avoidance. Although, some contradictory results have been reported, this could be related to the different species used, the specific times in development in which animals were exposed to stress, and differences in housing conditions.

In relation to behaviors related to anxiety in the elevated plus maze and open field, it has been observed increased locomotion and mixed results in relation to time and entries in the open arms. In particular, Sprague-Dawley rats exposed to social-defeat in early adolescence and tested in late adolescence (P56) in an elevated plus maze, spent more time in the open arms, and showed higher levels of locomotion compared to control rats (Watt, Burke, Renner, & Forster, 2009). On the other hand, CD1 male mice exposed to chronic social stress in early adolescence and tested as adults (P77), showed fewer entries in the open arms, less head-dips, and spent less time in the open arms compared to controls (Sterlemann, et al., 2008; Schmidt, et al., 2007). Similar results have been observed in adolescent male c57BL/6J mice exposed to social-defeat for 2 weeks. When tested as adults (after 3 weeks of stress exposure), previously defeated animals showed lower percentages of open-arm entries, less time spent in open arms, and higher percentage of closed-arm entries compared to controls (Kovalenko, et al., 2014). In the same way, Long-Evans rats exposed to social stress in mid adolescence and tested at P70, spent less time in the open arms than control animals, but the difference only approached significance. Additionally, chronic socially stressed animals had higher total locomotor activity than control animals (McCormick, et al., 2008). Nevertheless, single-housed Wistar rats exposed to social defeat and restrain stress in adolescence did not differ from control animals in the elevated plus maze (Bourke & Neigh, 2011). Nonetheless, it is important to mention that in some of these studies animals were tested during the light phase of the cycle.

The long-term effects of social stress in adolescence have also been evaluated in an open-field test. Sprague-Dawley rats exposed to social-defeat in early adolescence and tested in late adolescence (P56) showed higher levels of locomotion compared to control animals (Watt, et al., 2009). On the other hand, CD1 male mice exposed to chronic social stress starting between P26 and P28 (early adolescence) and tested as adults (P77), showed increased locomotion in the first five minutes of the test, but did not differ from control animals in other measures on the open field test (Sterlemann, et al., 2008; Schmidt, et al, 2007). Opposite to increased locomotion, adolescent male c57BL/6J mice (4-week-old) exposed to social-defeat for 2 weeks showed higher latency time of first movement from the center, compared to controls (Kovalenko, et al., 2014).

In a separate study, in order to study the capacity of animals to control their levels of anxiety in different contexts, the authors tested Wistar rats for contextual fear against the need to drink water induced by water deprivation. In this case, Wistar rats exposed to social defeat at P45 and P46 and tested in late adolescence (P68) in a water conflict test, showed decreased water consumption, less time drinking and higher latency to drink when tested in the defeated context, compared to controls. When animals were tested in an unfamiliar context or in the home cage, there were no differences with controls in any of the measures. Thus, it was observed that the effects of social defeat prolong to adulthood and is specific to defeat context (Vidal, et al., 2011a). In a separate study, animals were tested for defeat context anxiety by evaluating animals when exposed to cages in which adolescent defeat occurred, but without the larger resident male present. In this case, Sprague-Dawley exposed to social defeat in early adolescence and tested in late adolescence showed reduced active exploration behaviors such as locomotion, digging, and

substrate sniffing, and increased risk assessment behaviors than controls (Watt, et al., 2009).

These anxiety-like behaviors have also been tested in the novelty-induced suppression feeding test. In this test, animals encountering a desirable food in a novel environment will consume very limited quantities. In this case, CD1 male mice exposed to chronic social stress in early adolescence and tested as adults (P77), showed longer time than controls to initiate food consumption in a new environment (Schmidt, et al., 2007). Nevertheless, this effect was not observed when animals were tested 12 months after the stress exposure (Sterlemann, et al., 2008).

Using the defensive burying test to evaluate animals' response to a threatening or noxious object, it has been observed decrease in the active burying behavior. In particular, Sprague-Dawley rats exposed to social-defeat in early-adolescence and tested in a defensive burying test in adulthood (P70), showed decrease burying duration compared to controls, showing an inhibition of proactive behaviors (Bingham, et al., 2011).

The effects of socials tress have also been evaluated in the sucrose-preference test. This test has been used as an indicator of anhedonia, a state characterized by inability to experience pleasure related to depression. In this case, single-housed Wistar rats exposed to stress and restrain stress in adolescence did not differ from control animals when tested as adults (Bourke & Neigh, 2011). Similarly, in a test for depressive-like states such as the forced-swim test, single-housed Wistar rats exposed to social defeat and restrain stress in adolescence did not differ from control animals in any of the measures of the forced swim test (Bourke & Neigh, 2011).

Effects of social stress in social interaction have shown that c57BL/6J mice exposed to social-defeat in early adolescence and tested as adults, spent more time near an

unfamiliar animal, than an empty cylinder regardless of stress condition (Kovalenko, et al., 2014). In contrast, male c57BL/6J mice exposed to social defeat in early adolescence and tested as adults, showed social avoidance which was indicated by a significantly lower social interaction ratio in the adult mice (Zhang, Yuan, Shao, & Wang, 2016). In the same way, social avoidance has been observed in Wistar rats exposed to social defeat in mid-late adolescence, when tested as adults (P78) (Vidal, Buwalda, Koolhaas, 2011b; Vidal, et al., 2007).

The effect of social stress in memory have evaluated in object recognition test. In this case, Long-Evans rats exposed to social stress in mid adolescence, and tested at P70 in object recognition memory task, and an object location task, showed that whereas control animals spent more time investigating the object in the novel location, previously stressed animals did not discriminate between novel and familiar object. On the other hand, in the object recognition test, all animals spent more time investigating the novel than the familiar object irrespective of stress group. Thus, exposure to social instability stress during adolescence alters memory performance specific to a hippocampal-dependent task (McCormick, et al., 2012).

In relation to long-term consequences of social stress in aggression, it has been observed that male golden hamsters exposed to social-defeat from P28 to P42 (early adolescence), and tested at P70 in the presence of a smaller and younger animal, previously defeated animals were more likely than controls to be aggressive toward the intruders, showing higher number attacks. Additionally, the exposure to defeat during adolescence has been shown to alter the development of agonistic behavior, specifically, accelerating the transition from play fighting into adult aggression (Wommack, et al., 2003). The same increased aggression in adulthood has been observed in c57BL/6J mice exposed to social

defeat during early adolescence (4 weeks old), evidenced by shorter latencies of the first attack, and higher total hostile behavior, including attacks, digging and aggressive grooming, compared to control animals (Kovalenko, et al., 2014). In the same way, increased aggression in adulthood has also been observed in Wistar Hans rats exposed to variable stress from P28 to P42 and tested as adults. This has been evidenced by an increased in the amount of time spent keeping down the intruder compared with controls (Tzanoulinou, Riccio, de Boer & Sandi, 2014; Márquez, et al., 2013). Although the stress procedure used was not social, it is important to highlight the effect of exposure to stress during adolescence and increased aggression during adulthood.

Translational significance of early stress exposure

In humans, it has been observed that childhood trauma (e.g. emotional neglect, psychological, physical or sexual abuse) appears to be an important risk factor for depression, anxiety disorders, personality disorders, PTSD, and drug abuse in adulthood (Hovens, Wiersma, Giltay, van Oppen, Spinhoven, Penninx, Zitman, 2010; De Venter, Demyttenaere, Bruffaerts, 2013; Martín-Blanco, et al., 2016; De Bellis, 2002). In the same way, exposure during childhood to bullying (as a behavior that is intentional, repeated, and involves a power imbalance), is related to higher rates of depression, anxiety, panic attacks, suicidal thoughts and behaviors during adulthood as well (Newman, Holden, & Delville, 2005; Silberg, Copeland, Linker, Moore, Roberson-Nay, & York, 2016; Reid, Holt, Bowman, Espelage, & Green, 2016).

SOCIAL STRESS IN ADULTHOOD

In general adults exposed to social stress have shown decrease exploration of the open arms in an elevated plus maze, decrease locomotion, decrease exploration in the defat

context, social avoidance and lack of aggression in the presence of any intruder. Wistar rats exposed to a single social-defeat at P90 and evaluated at P104, showed a decrease in the percentage of the time spent in the open arms of an elevated plus maze, especially if animals were single-housed after the defeat episode (Nakayasu & Ishii, 2008). On the other hand, male adult Tryon Maze Duli S1 rats (known for its high level of social activity) exposed to one or two social-defeat episodes and tested in an open field, showed decreased locomotor activity two days after the defeat compared to controls (Meerlo, Over, Daana, Van den Hoofdakker, & Koolhaas, 1996).

Long-term effects of social stress exposure in adolescence			
Species	**Test**	**Effect**	**Reference**
Sprague-Dawley	EPM	More time in open arms, higher levels of locomotion	Watt, et al., 2009
CD1	EPM	Fewer entries in open-arms, less head-dips and less time in open arms	Sterlemann, et al., 2008; Schmidt, et al., 2007
c57BL/6J	EPM	Lower percentages of open-arm entries, less time in open arms, and higher percentage of closed-arm entries	Kovalenko, et. al., 2014
Long-Evans	EPM	Lless time in open arms, higher total locomotor activity	McCormick, et al., 2008
Wistar	EPM	=	Bourke, & Neigh, 2011
Sprague-Dawley	Open-field	Higher levels of locomotion	Watt, et. al., 2009
CD1	Open-field	Higher levels of locomotion	Sterlemann, et. al., 2008; Schmidt, et. al, 2007
c57BL/6J	Open-field	Higher latency time of first movement from the center	Kovalenko, et. al., 2014
Wistar	Water conflict	Decreased water consumption, less time drinking and higher latency to drink when tested in the defeated context. No effect when tested in unfamiliar contex or home-cage	Vidal, et al., 2011a
Sprague-Dawley	Defeat context anxiety	Reduced locomotion, digging, and substrate sniffing. Increased risk assessment behaviors	Watt, et. al., 2009
CD1	Novelty-induced suppression of feeding	Longer time to initiate food consumption in a new environment	Schmidt, et. al., 2007
Sprague-Dawley	Defensive burying	Decrease burying duration	Bingham, et. al., 2011
Wistar	Sucrose preferece	=	Bourke, & Neigh, 2011
Wistar	Forced swim	=	Bourke, & Neigh, 2011
c57BL/6J	Social interaction	More time near an unfamiliar animal	Kovalenko, et. al., 2014
c57BL/6J	Social interaction	Social avoidance	Zhang, et. al., 2016
Wistar	Social interaction	Social avoidance	Vidal, et al., 2011b; Vidal, et al., 2007
Long-Evans	Object recognition	Same time between novel and familiar object in novel location. = when object was new	McCormick, et. al., 2012
Hamster	Aggression	Higher number of attacks toward a younger animals. Faster transition into adult aggression	Wommack, et al., 2003
c57BL/6J	Aggression	Shorter latencies of the first attack, and higher total hostile behavior, including attacks, digging and aggressive grooming	Kovalenko, et. al., 2014

Table 4. Long-term effects of social stress exposure in early adolescence. Only studies using males are including. EPM: Elevated-Plus-Maze. = no significant difference with control group.

In a different test, Sprague-Dawley rats exposed as adults to three days of social-defeat and evaluated in the same defeat context, showed decreased rearing exploration, and increased risk assessment when compared to controls (Razzoli, Carboni, Guidi, Gerrard, & Arban, 2007).

Social avoidance has also been observed in adults after social stress exposure. Adult Wistar rats exposed to a single social-defeat showed increased social avoidance, when tested up to 10 days after the stress exposure (Haller & Bakos, 2002). In the same way, male C57BL/6J mice exposed to social defeat for 9 days at P70 and tested six weeks later, showed social avoidance evidenced by a significantly lower social interaction ratio compared to control mice (Zhang, et al., 2016). Likewise, Male adult Tryon Maze Duli S1 rats exposed to one or two social-defeat episodes and evaluated in social interaction test, showed social avoidance toward unfamiliar nonaggressive conspecifics two days after the defeat compared to controls (Meerlo, et al., 1996).

The effect on aggression of social stress in adulthood has also been studied. Adult male golden hamsters exposed to social-defeat display submissive behavior and lack of aggression in the presence of any intruder, regardless of size and age. This behavioral effect has been called conditioned-defeat and has also been observed in rats (Potegal, et al., 1993) and mice (Frischknecht, Siegfried, & Waser, 1982). In adult male Syrian hamsters this has been observed by placing the animals in the cage of a resident during 5 min for 4 consecutive days, or for 5 min four times in 1 day, and tested the day after the last stress exposure in the presence of a non-aggressive animal. When tested with small nonaggressive opponents, previously stressed animals failed to attack the intruders, displayed submissive/defensive behaviors and decreased territorial aggression, despite the

fact that the intruder animals exhibited non-aggressive behavior (Potegal, et al., 1993; Jasnow, et al., 1999).

Thus, one key differential effect of social stress exposure in adolescence and adulthood relates to aggression. As previously mentioned, adolescent male golden hamsters exposed to social-defeat showed short-term context-dependent alteration of aggressive behavior. Specifically, defeated animals are more likely to be aggressive toward smaller, younger and submissive animals. On the other hand, defeated animals are less likely to attack intruders that are the same age and weight (Delville, et al., 1998). These defeated animals in the presence of a smaller and younger animal when tested as adults (P70), showed increased aggressive toward intruders (Wommack, et al., 2003). Contrary to this, when adult male hamsters are exposed social-defeat, the complete opposite is observed. These defeated adults display submissive behaviors and lack of aggression in the presence of any intruder, regardless of size and age (Potegal, et al., 1993; Jasnow, et al., 1999).

RECURRENT SOCIAL STRESS IN ADOLESCENCE AND ADULTHOOD

Another possibility is that exposure to social stress extends from adolescence into adulthood. In this case, male hamsters exposed to social defeat from P26 to P38 showed enhanced aggression when tested at P48. After becoming adults these animals were exposed to a novel cage for12 days at P86, or to social defeat for the same period of time at P146. When tested after the novel cage exposure, these animals showed the same pattern of enhanced aggression than when tested at P48 after stress. This is, animals exposed to socials stress in adolescence show as adults enhanced aggression evidenced by shorter latencies to attack, and higher number of bites and flank marks, compared to animals that

were exposed to social stress for the first time as adults. When animals exposed to social stress in adolescence were exposed to stress again as adults (P146), they showed the same pattern of decreased aggression observed in animals exposed to social stress in adulthood only, evidenced by significant decreased number of bites and flank marks, and increased latency to attack. This study showed that while adolescent subjugation favored future enhanced aggression, it did not protect these animals from the behavioral consequences of losing fights as adults. This is, when exposed to social stress in adolescence and adulthood, these animals showed the same pattern of stable, submissive behavior observed in animals exposed to social stress only as adults (Ferris, Messenger, & Sullivan, 2005).

Agonistic behavior and aggression

Agonistic behavior refers to all behaviors related to social interactions between conspecifics. When an individual encounter another, they may engage in a series of behaviors such as flank marking, auditory signals, and threats that are displayed to establish dominant/subordinate relationships, or to confirm the ownership of a territory. Depending of factors such as gender, body weight and social experience of the conspecific, these social interactions can change, and attacks and bites may be displayed, initiating an aggressive interaction (Pfaff, Arnold, Etgen, Fahrbach, & Rubin, 2002).

AGONISTIC BEHAVIORS AND ADOLESCENCE

Agonistic behavior changes during development. The first agonistic behavior observed, described as play fighting, involves one animal approaching and pouncing upon his opponent, which results in both animals wrestling in the on-back and on-top positions (Grant & Chance, 1958; Bolles, & Woods, 1964; Baenninger, 1967). In male Long-Evans rats play-fighting peaks at P30, and declines abruptly as animals undergo adolescence until

P60, where it continues gradually decreasing until P90 (Takahashi & Lore, 1983). On the other hand, hamsters engage in play fighting with their littermates as soon as they develop motor coordination around P20 (Goldman & Swanson, 1975; Siegel, 1985). This behavior continues during adolescence, and peaks during early adolescence (P33), then steadily declines until stabilizing during the second half of adolescence (starting around P47) when it starts changing into adult aggression. In hamsters, the transition from play fighting to aggression during adolescence is marked by reduced attack frequency, and a shift from the target of the attacks based on the location of the sent glands from the face, to attacks directed at the flanks, and later to the lower belly and rump (Wommack, et al., 2003).

This maturation of agonistic behavior in hamsters is affected by exposure to social stress in adolescence. In males, animals exposed to social defeat, the transition from face attacks to lower belly/rump attacks is accelerated (Wommack, et al., 2003). This acceleration is cortisol-dependent, as treatment with corticosteroids accelerated this maturation in the absence of stress, and blockade of corticosteroid receptors type II delay this maturation (Wommack & Delville, 2007). Furthermore, the gradual reduction of attack frequency during adolescence is also affected by exposure to social stress, as defeated animals maintain a higher level of attack frequency into adulthood (Wommack, et al., 2003).

AGGRESSION

In animals and in humans there are different forms of aggression, nevertheless the subtypes of aggression differ between humans and animals. In humans, the behavior has been separated into proactive and reactive aggression. Proactive aggression is characterized as instrumental, or premeditated, and is associated with low emotional reactivity, decreased

sympathetic nervous system, and decrease HPA axis responsiveness. Reactive aggression on the other hand, is characterized as impulsive and hostile, and is associated with heightened emotional reactivity, elevated sympathetic nervous system, and elevated HPA axis responsiveness (Cervantes & Delville, 2007; Veenema, 2009; Kockler, Stanford, Nelson, Meloy, & Sanford, 2006; Haller, van de Schraaf, & Kruk, 2001).

In animals on the other hand, aggression has been defined as behavior observed under different contexts (Moyer, 1968), but also as specific behavioral responses to an opponent (Brain, 1979; Blanchard & Blanchard, 1977). Based on the context in which aggression is observed, seven types of aggression can be identified: spatial aggression (territoriality), aggression over food or other ingestive resource, aggression over dominance status, sexual aggression, parental aggression, antipredator aggression (and interspecific aggression), and irritable aggression (Moyer, 1968). Based on the responses displayed during an agonistic encounter, such as behavioral sequences and targets of attacks, three basic types of aggression are identified: offensive, defensive, and injurious aggression, which has elements of offensive and defensive aggression.

Offensive aggression is usually displayed by adult males when competing for or protecting resources and utilities like food, territory, and mating. It is also used to establish dominant/subordinate relations and is characterized in rats by non-lethal location bites such as the middle of the back. Defensive aggression is used as a protective response displayed by animals that are attacked by a conspecific or by a predator, and is characterized by bites on the face or the most threatening body part of an attacker. Injurious aggression, is performed to incapacitate the opponent, and it is displayed by mothers protecting their offspring (maternal aggression) and also by animals when attacking preys. It is characterized by attacks aimed to provoke injuries in highly vulnerable targets of the

opponent, such as throat and belly (Adams, 2006; Cervantes & Delville, 2007; Blanchard & Blanchard, 1977, Blanchard & Blanchard, 1988; Blanchard, et al., 2003).

Although context-based models of aggression are useful because they point out the extent of contexts in which aggression may be expressed, they leave out the dynamics of aggressive encounters. In most cases, these encounters include two organisms that may differ in species, gender, experience, and physiological variables, which can determine the behaviors displayed by animals with different functions or outcomes. Therefore, response-based models are most useful in animals for neurobiological studies. It is also important to mention, that response-based models of aggression have been useful for definition of human aggression and allow translational studies by looking at common features observed in the different types of aggression. Specifically, proactive aggression shows parallels with injurious aggression in animals, since it is associated with inhibited emotional reactivity, and goal directed behaviors, while reactive aggression may show parallels with offensive aggression in animals, since it is associated with enhanced emotional reactivity and impulsivity (Cervantes & Delville, 2007; Kockler, et al., 2006; Haller, et al., 2001).

Impulsivity and aggression

As explain earlier, aggression has been associated with impulsivity under reactive aggression in humans. Furthermore, aggression and impulsivity are traits of several mental disorders (Brevet-Aebya, Brunelina, Icetaa, Padovanc, & Poulet, 2016). In animals, offensive aggression also includes an association with impulsivity. Specifically, individuals performing consistently repeated attacks on intruders in a resident/intruder test have also been characterized as impulsive, as these animals prefer small immediate rewards over larger but delayed ones on a delay discounting task (Cervantes & Delville, 2007;

Cervantes & Delville, 2009). These animals are also more likely to maintain a high rate of lever pressing under long delays to get rewards, while their brains present widespread pCREB activation, a possible sign of elevated emotional reactivity (David, Cervantes, Trosky, Salinas & Delville, 2004). Such findings linking aggression with impulsivity in animals have been supported by later studies in rats through a variety of testing procedures (Coppens, de Boer, Buwalda, & Koolhaas, 2014; Fodor, et al., 2014; Cooper, Goings, Kim, & Wood, 2014; Wallin, Alves, & Wood, 2015).

While some studies have associated aggression with impulsivity, it is important to note that impulsivity is not a unitary construct. There are at least two behavioral expressions of impulsivity: impulsive action and impulsive choice. Impulsive action refers to the inability to inhibit a previously rewarded response. This failure in inhibition can be observed as failure to withhold, stop or postpone a motor response (action inhibition and waiting) (Bari & Robbins, 2013). Impulsive choice reflects decision making, and is characterized by a preference for suboptimal selections that include the preference for immediate smaller rewards over more beneficial but delayed, higher effort or less risky ones (Bari & Robbins, 2013).

Impulsive action and impulsive choice can be evaluated using different paradigms. Impulsive action is commonly tested through Go-NoGo task and stop signal task (SST) for action inhibition, and five-choice serial reaction time task (5-CSRTT) and Differential Reinforcement of Low Rates (DRL) task for waiting. In the Go-NoGo task some trials present a Go cue, and a subset of trials preset a NoGo cue, which signals that subjects must withhold their response (Newman, Widom, & Nathan, 1985), and failure to withhold responding is an indicator of impulsive action. On the other hand, the stop signal task requires the subjects to respond as fast as possible to a Go signal, and in a subset of trials,

a stop signal is presented with varying delays after the Go signal. Subjects are required to inhibit responding at different stop signal delays, thus this task indicates how capable are the subjects of cancelling an action once it has already been initiated (Logan, Schachar, & Tannock, 1997). The 5-CSRTT evaluates the waiting component of impulsive action. This task requires animals to wait and detect a light presented in one of five holes, and to make a nose-poke response in the correct spatial location to receive a food reward. Premature responses (made before the presentation of the stimulus) are regarded as a form of impulsive behavior (Robbins, 2002). Finally, in the DRL task, reinforcement is contingent upon responses which are spaced for a specific amount of time from the previous response (Kramer & Rilling, 1970). Thus, the DRL task requires the subject to pause for a specified minimum period between responses to obtain a reward, failure to do so indicates impulsivity.

Impulsive choice is usually evaluated with delay, effort or probability discounting paradigms. In a typical delay discounting procedure, subjects need to make a series of choices between responses leading to large delayed rewards, or responses leading to smaller immediate rewards (Ainslie, 1975). Greater discounting of the large reward (e.g., steeper discounting) is indicative of more impulsive behavior. In effort discounting, subjects must choose between a small reward that can be easily obtained and a large reward that requires greater effort to obtain (Cousins, Atherton, Turner, & Salamone, 1996). Finally, in a probability discounting procedure subjects need to choose between responses that lead to a small reward that is delivered with greater certainty, and responses that lead to a larger, more uncertain reward that is delivered according to various probabilities (Rachlin, Raineri, & Cross, 1991).

TOWARD A NEW MODEL OF AGGRESSION

As it has been shown, aggression has been associated with impulsivity, but these associations involve different forms of impulsivity making the relation between them ambiguous. For example, testosterone administration enhances aggression in humans and animals (Midgley, Heather, & Davies, 2001; Wood, et al., 2013). Additionally, testosterone-treated rats show decreased impulsive choice, and enhanced risk raking (Wood, et al., 2013), without differing in a Go-NoGo task compared to vehicle-treated controls (Cooper, et al., 2014). On the other hand, inherently aggressive hamsters prefer small immediate rewards over larger but delayed ones on a delay discounting task (Cervantes & Delville, 2007; Cervantes & Delville, 2009). Additionally, when testing two rats with different emotional profiles, selected for rapid (Roman-high avoidance, RHA) vs extremely poor (Roman-low avoidance, RLA) acquisition of a two-way active avoidance in the shuttle box, RLA show a tendency toward a higher level of offensive aggression, in addition to decreased impulsive behavior compared to RHA when tested in impulsive choice, 5-CSRTT, and a variable-interval 15 (VI-15) schedule of reinforcement, (Coppens, de Boer, Steimer, & Koolhaas, 2012; Moreno, et al., 2010). Thus, these different forms of impulsivity observed in aggressive animals, suggest the possibility of multiple aggressive/impulsive profiles and possibly differing neurobiological backgrounds.

In humans the relation between aggression and impulsivity has been observed in mental disorders. Borderline personality disorder (BPD) is characterized by aggression and impulsivity (Skodol, et al., 2002). Specifically, some studies have found enhanced impulsive choice in a delay discounting task (Lawrence, Allen, & Chanen, 2010), while others have not (Dougherty, Bjork, Huckabee, Moeller, & Swann, 1999). Furthermore, BPD patients made more errors during NoGo trials in a Go-NoGo task, and showed shorter

reaction times in both Go and NoGo trials (Rentrop, et al., 2008) suggesting enhanced impulsive action. Similarly, Bipolar disorder is also characterized by impulsivity and aggression (Látalová, 2009). In particular, bipolar patients show greater preference for immediate, smaller rewards over larger, delayed rewards relative to healthy individuals, suggesting impulsive choice (Ahn, et al., 2011). Also, these individuals had more difficulty inhibiting motor responses, evidenced by a marginally higher error rate in a Go-NoGo task (Fleck, et al., 2011). Additionally, bipolar subjects show longer stop times in a SSRTs, in addition to more impulsive responses and slower reaction time in a Delay Reward Task (Strakowski, Fleck, DelBello, Adler, Shear, Kotwal, & Arndt, 2010). Nevertheless, one difference between BPD and Bipolar seems to be perseverance. Some studies have shown differences in perseverance between these psychopathologies (Bøen, et al., 2015), while some others have not (Shafiee-Kandjani, et al., 2017). This lack of consistency suggests the existence of different subtypes of aggressive/impulsive profiles in humans.

The fact that different forms of impulsivity are related to aggression, raise the question about what form of impulsivity is associated with the enhanced aggression observed after stress exposure, especially since they involve different neural circuits and different neurotransmitter systems. However, since impulsivity and aggression are not unitary concepts, it is possible that there is more than one impulsive/aggression profile. Perhaps, the concept of aggression should be re-analyzed, and include different subtypes of offensive aggression based on their relation with impulsivity, or perhaps the concept of aggression needs to be redefined as part of an interacting multidimensional construct mediating personality. As such, the studies proposed in this work will help in the attempt to redefine aggression, and will help define the forms of impulsivity associated with

enhanced aggression caused by early stress exposure, suggesting possible specific brain areas related.

Neural mechanisms of impulsivity

Impulsivity is likely controlled through neural networks involving the prefrontal cortex (PFC), especially its connection with anterior cingulate cortex, orbitofrontal cortex, and infralimbic cortex, striatum, raphe nuclei, ventral tegmental area, and nucleus accumbens (Dalley, Mar, Economidou, & Robbins, 2008). However, as previously mentioned, there are different forms of impulsivity, therefore, there are also different neural networks associated with them.

ACTION INHIBITION

Performance in tasks involving action inhibition such as SSRT and Go-NoGo is related to activity in the orbitofrontal cortex, medial striatum, and subthalamic nuclei. In monkeys, lesions in the inferior frontal convexity (IC) an area of the prefrontal cortex adjacent to the orbitofrontal cortex (homologue of inferior frontal cortex in humans), results in impairments in the ability to inhibit the response during NoGo trials in a Go-NoGo task (Iversen, & Mishkin, 1970). In the same way, studies with neuroimaging have shown that bilateral middle and inferior frontal gyri, anterior cingulate, anterior insula, pre-supplemental motor area (pre-SMA), inferior parietal cortex, and thalamic regions are common areas activated during inhibition in Go-NoGo and SSRT tasks (Rubia, et al., 2001; Dambacher, et al., 2014). Additionally, it has been observed that during the SST task, the stop process activates basal ganglia (globus pallidum), and subthalamic nucleus (STN) (Aron & Poldrack, 2006). Moreover, animal studies have shown that bilateral lesion in the

medial prefrontal cortex, and nucleus accumbens core have no effect on stop accuracy, or inhibition performance in a SSRT task in rats (Eagle & Robbins, 2003) (Figure 1).

WAITING IMPULSIVITY

On the other hand, in tasks with a waiting component in which animals need to withhold responding until it is required, such as 5-CSRTT, performance is related to activity to medial PFC, specially infralimbic cortex (IL), medial and lateral striatum, and shell of nucleus accumbens (NAc). Excitotoxic lesions with quinolinic acid in orbitofrontal cortex (OFC), infralimbic cortex (IL) or anterior cingulate (ACC) in rats, showed that lesions in IL resulted in increased premature responses in a 5-CSRTT, while lesions in OFC predominantly induced perseverative responses. Lesions of the lateral and ventral striatum lead to a severe impairment in the 5-CSRTT, marked by a significant increase in omissions requiring retraining of the task, but without an increase in premature responses. In contrast, lesions of the medial striatum lead to premature and perseverative responses (Rogers, Baunez, Everitt, & Robbins, 2001). Finally, microinjections of the GABA-A receptor agonist muscimol into the nucleus accumbens shell, lead to an increase in premature responses. In contrast, injection in nucleus accumbens core decrease accuracy and increased omissions, but without affecting premature responses (Feja, Hayn, & Koch, 2014) (Figure 1).

IMPULSIVE CHOICE

Finally, performance in impulsive choice tasks, such as delay discounting, mainly involve the basolateral amygdala, NAc core and hippocampus. Lesions of the NAc core with quinolinic acid induced a profound and lasting effect in rats' ability to choose the bigger delayed reward, evidencing increase impulsivity. On the other hand, lesions in

anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC) did not affect the preference for the delayed reward (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001). Bilateral lesions with quinolinic acid of the basolateral amygdala (BLA) increase impulsive choice, evidence by a preference of a small immediate reward over a bigger but delayed one. On the other hand, lesions of the orbitofrontal cortex (OC) improve impulsive choice by increasing the choice of the larger delayed reward (Winstanley, Theobald, Cardinal, & Robbins, 2004). Finally, lesions of the dorsal and ventral hippocampus with N-methyl-D-aspartic acid increase impulsive choice (Cheung & Cardinal, 2005; Mariano, et al., 2009), while other studies have found that lesions of the orbitofrontal cortex did not have an effect (Mariano, et al., 2009).

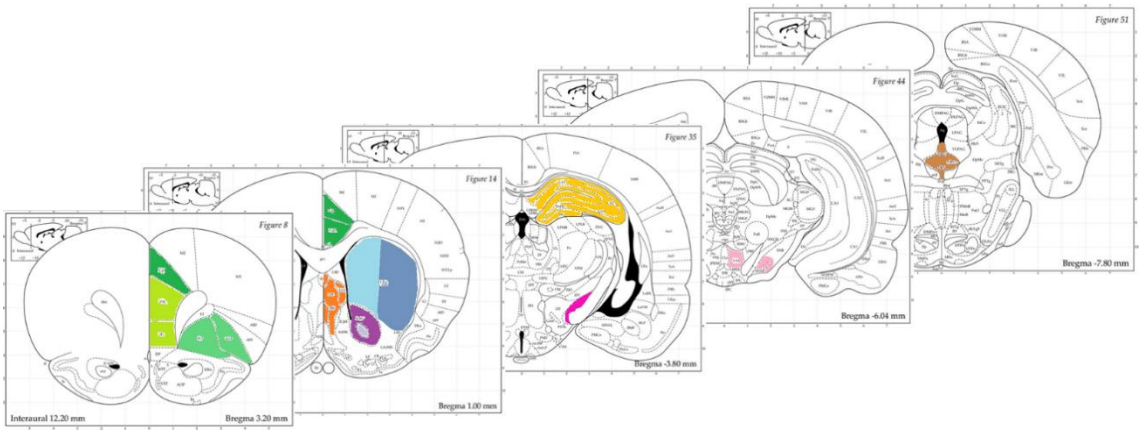


Figure 1. Representation of structures involved in control of action inhibition and waiting impulsivity. Adapted from: The rat brain in stereotaxic coordinates: hard cover edition. Access Online via Elsevier, 2006. In green: IFG (inferior frontal gyrus), OFC (orbitofrontal cortex), ACC (anterior cingulate), IL (infralimbic cortex). In orange: Medial and lateral septum. In light purple: NAc-core (Nucleus accumbens core), dark purple: NAc-s (Nucelus Accumbens shell). In blue: Caudate/Putamen. In fuchsia: STN (subthalamic nuclei). In yellow: HC (Hippocampus). In pink: SNc (Substantia Nigra) and VTA (Ventral tegmental area). In brown: Raphe nuclei

NEUROCHEMICAL BASES OF IMPULSIVITY

Two major neurotransmitter systems have been associated with impulsivity: Dopamine (DA) and Serotonin (5-HT).

Dopamine (DA)

Evidence of the role of DA in impulsivity in humans originates from studies with patients with Parkinson's disease (PD) and attention deficit hyperactivity disorder (ADHD). In PD it has been observed a small proportion of patients which develop compulsive gambling, hypersexuality and binge eating after the administration of DA replacement. On the other hand, studies with ADHD have shown that impulsivity in these individuals is related to lower DA in the striatum (Dalley & Roiser, 2012).

Dopamine and action inhibition

Administration of d-amphetamine has been used to study the role of DA in impulsivity. Rats injected with d-amphetamine intraperitoneally and evaluated in a SSRT task showed a dose dependent decrease in stop reaction time in slow stoppers, without an effect on fast stoppers, suggesting an important role of DA in impulsive action (Feola, de Wit, & Richards, 2000). In the same way, administration of d-amphetamine in healthy subjects showed a significant reduction in stop reaction time in slow stoppers, compared to placebo. Additionally, there was no effect on Go reaction time, indicating that the drug affected response inhibition specifically and not overall reaction time. Furthermore, it has also been observed a decreased in responses during NoGo trials in a Go-NoGo task. Specifically, d-amphetamine reduced NoGo responses in subjects with high rate of these responses, while no effect was observed in subjects with low rates of NoGo responses (de Wit, Enggasser, & Richards, 2002). The same effect has been observed in children with

ADHD, where after treatment with methylphenidate and tested in a stopping task, it was observed higher inhibition function when compared to placebo (Tannock, Schachar, Carr, Chajczyk, & Logan, 1989).

It has been observed that in a SSRT task inhibition is modulated by dopamine in the striatum, but it is receptor-specific and anatomically constrained. Dopamine D1 and D2 receptors in the dorsomedial striatum, but not in nucleus accumbens core, had opposing functions. D1 receptor antagonist SCH 23390 decreased stop times in SSRT, allowing faster inhibition. Conversely, D2 antagonist sulpiride increase the stop time, suggesting that dopamine acting in D2 receptors makes more likely that a response is inhibited (Eagle, Wong, Allan, Mar, Theobald, & Robbins, 2011). Additionally, the ventral striatum and the availability of D2/3 receptors have shown an inverse relationship with impulsivity, in particular with impulsive action (Dalley, et al., 2007). Therefore, it seems that the role of Dopamine on impulsive action is receptor and site-specific

Dopamine and waiting impulsivity

Contrary to the effects on action inhibition, stimulants impair the capacity of individuals to wait in the 5-CSRTT. Rats injected with d-amphetamine and evaluated in a response inhibition task in which animals need to wait for a signal in order to make a response and be rewarded, showed differential effects according to the predictability of the delay. For this, separate groups were trained with fixed or variable delays. Animals injected with d-amphetamine significantly increase premature responses when the delay was fixed, and decreased premature responses when the delay was variable and unpredictable. The effect of d-amphetamine on this task depends on whether the animals can time the delay interval, suggesting that amphetamine affects some other process such as timing or

attention, which has differential effects on premature responding in these different conditions (Hayton, Maracle, & Olmstead, 2012). In a separate study where animals were treated with amphetamine, cocaine, or nicotine and evaluated in a 5-CSRTT, it was observed that premature responses were increased with all three treatments. Additionally, systemic treatment with the dopamine D1 receptor antagonist SCH 23390 dose-dependently decreased premature responding, while D2 receptor antagonist eticlopride did not. When animals were injected with a high dose of SCH 23390 after administration of amphetamine, cocaine and nicotine, their effect on premature responding were reduced. Furthermore, treatment with the dopamine reuptake inhibitor GBR 129090 enhanced premature responding at the higher doses (van Gaalen, Brueggeman, Bronius, Schoffelmeer, & Vanderschuren, 2006).

In the same way, eticlopride a D2 antagonist did not affect the premature responses in a 5-CSRTT when infused into the nucleus accumbens core or shell. On the other hand, infusion of the D1 antagonist SCH 23390 decreased the number of premature responses with no differences between site of infusion (core or shell). These data suggest that the waiting component of inhibitory control processes are under control of tonic activation of dopamine D1 rather than dopamine D2 receptors in the nucleus accumbens core and shell (Pattij, Janssen, Vanderschuren, Schoffelmeer, & van Gaalen, 2007). Other studies have observed that higher D1 mRNA expression in the NAc-shell predicts greater impulsive action (waiting component) in a Differential Reinforcement of Low Rates of Responding task, whereas lower D2 mRNA expression in the NAc-core predicts greater impulsivity in this task (Simon, et al., 2013). Nonetheless, additional evidence has indicated that D2/D3 receptor are lower in NAc-shell, while D1 are lower in NAc-core in high impulsive animals evaluated in a 5-CSRTT (Jupp, et al., 2013). Finally, a dopamine transporter knock-in mice

(DAT Val559) evaluated in different impulsivity tasks exhibited impulsive action in a 5-CSRTT, without showing deficits inhibiting pre-motor responses in a Go-NoGo task. (Davis, et al., 2018).

Dopamine and impulsive choice

Dopamine has also been associated with impulsive choice. Administration of d-amphetamine to healthy subjects showed decreased delay discounting, namely, after administration of the drug subjects showed a less steep discounting of delayed rewards compared to placebo (de Wit, et al, 2002). In the same way, rats injected with amphetamine or methylphenidate and tested in a delay discounting task showed decreased impulsivity, as evidenced by an increased the choice of the large delayed reward in animals treated with the stimulants. Additionally, selective inhibition of the dopamine reuptake transporter with GBR 12909 also increased the choice for large delayed reinforce in the highest dose, compared to saline (van Gaalen, van Koten, Schoffelmeer, & Vanderschuren, 2006). Furthermore, co-administration of dopamine antagonist flupenthixol with d-amphetamine did not decrease impulsive choice (Winstanley, Dalley, Theobald, & Robbins, 2003). Finally, evaluation of impulsivity through an inter-temporal choice behavior (choice between rewards differing in size and delay) after depletion of dopamine in orbitofrontal cortex with 6-hydroxydopamine in female rats, showed that dopaminergic afferents to the orbitofrontal cortex contribute to the regulation of impulsive choice, probably participating in the determination of the organisms' sensitivity to reinforce seize and delay (Kheramin, et al., 2004).

The specificity of D1 and D2 receptors in impulsive choice is less clear. Blockade of dopamine receptor D1 with SCH-23390 increased impulsivity at the 0s and 10s delay

with the highest doses. On the other hand, D2 receptor antagonist eticloripe did not affect impulsive choice (van Gaalen, et al., 2006). However, other studies have shown that D1 agonist SKF 81297 and antagonist SCH 23390 did not have an effect on impulsive choice, while D2 agonist quinpirole and antagonist eticlopride injected into the medial prefrontal cortex (mPFC) increased impulsive choice (Yates, et al., 2014; Zeeb, Floresco, & Winstanley, 2010).

Serotonin (5-HT)

Serotonin and action inhibition

To date, there is no clear effect of serotonin manipulations on action inhibition. Action inhibition tested through a SSRT task is not affected by intracerebroventricular (i.c.v.) administration of 5,7-dihydroxytryptamine (5,7-DHT), which leads to a ~80% depletion of serotonin (Eagle, et al., 2009). On the other hand, effects on Go-NoGo task showed that i.c.v administration of 5,7-DHT increased responses during NoGo trials compared to sham control animals, showing an increase in impulsive action (Harrison, Everitt, & Robbins, 1999).

Serotonin and waiting impulsivity

A role of serotonin on the waiting component of impulsive action has been observed. Depletion of forebrain 5-HT with i.c.v administration of 5,7-DHT produced a significant and long-lasting increase in premature responses in a 5-CSRT task (Harrison, Everitt, & Robbins, 1997b). In the same way, in a modified version of a SSRT task, in which an extended limited hold period was presented, animals treated with i.c.v. 5,7-DHT showed significant deficits in withholding the stop response (Eagle, et al., 2009). An

extended limited hold assesses the ability to withhold a response or "wait", therefore results from this modified version could be comparable with the ones from 5-CSRTT.

The dorsal raphe nucleus (DR), one of the largest serotonergic nuclei, has been extensively related to patience. Several studies have evidenced that 5-HT levels in the dorsal raphe significantly increase when animals wait for rewards (Miyazaki, Miyazaki, & Doya, 2011a; 2011b; Fonseca, Murakami, & Mainen, 2015).

Serotonin has a wide family of receptors, including 7 receptor families and several subtypes. The 5-HT2 receptor family (5-HT2A/2B/2C) is perhaps the most studied in relation to impulsive action, specifically, premature responding in 5-CSRTT. In general, it has been observed that 5-HT1 agonist and antagonist decrease the number of premature responses, evidencing enhanced impulsive control. On the other hand, 5-HT2A agonist and 5-HT2C antagonist seem to increase the number of premature responses, while 5-HT2A antagonist decreases the number of premature response (Table 5).

Receptor	Name	Administration	5-CSRTT	Reference
Agonist				
1A	DPAT	Prelimbic cortex	=	Winstanley, Chudasama, Dalley, Theobald, Glennon, & Robbins, 2003.
		Systemic	=	
	Tandospirone (partial	Systemic	-	Ohmura, Kumamoto, Tsutsui-Kimura, Minami, Izumi, Yoshida, & Yoshioka, 2013
Antagonist				
1A	WAY100635	Systemic	-	Ohmura, et. al., 2013
Agonist				
2A	DOI	Systemic	+	Koskinen, Ruotsalainen, & Sirviö, 2000
		Subcutaneus	+	Koskinen & Sirviö, 2001
		OFC+BLA	+	Hadamitzky & Koch, 2009
		Anerior cingulate cortex	=	Koskinen, Ruotsalainen, & Sirviö, 2000
		Systemic	=	Fletcher, Tampakeras, Sinyard, & Higgins, 2007
		Nacc(core/shell)	=	Koskinen & Sirviö, 2001
2C	WAY-163909	Systemic	-	Navarra, Comery, Graf, Rosenzweig-Lipson, & Day, 2008
2A,C	Ro60-0175	Systemic	=	Fletcher, Tampakeras, Sinyard, & Higgins, 2007
Antagonist				
2A	Ketansarin	Systemic	=	Koskinen, Ruotsalainen, & Sirviö, 2000
		Subcutaneus/mPFC	-	Passetti, Dalley, Robbins, 2003
		Systemic	-	Talpos, Wilkinson, & Robbins, 2006
		Systemic	-	Fletcher, Tampakeras, Sinyard, & Higgins, 2007
		OFC+BLA	=	Hadamitzky & Koch, 2009
2A	M100907	Systemic	-	Winstanley, Theobald, Dalley, Glennon, & Robbins, 2004
		Prelimbic cortex	-	Winstanley, Chudasama, Dalley, Theobald, Glennon, & Robbins, 2003.
		Systemic	-	Fletcher, Tampakeras, Sinyard, & Higgins, 2007
2B	SB215505	Systemic	=	Fletcher, Tampakeras, Sinyard, & Higgins, 2007
2C	SB 242084	Systemic	+	Winstanley, Theobald, Dalley, Glennon, & Robbins, 2004
		Systemic	+	Fletcher, Tampakeras, Sinyard, & Higgins, 2007
2B,C	SER 082	Systemic	=	Talpos, Wilkinson, & Robbins, 2006
Antagonist				
6	SB 271046-A	Systemic	=	Talpos, Wilkinson, & Robbins, 2006

Table 5. Serotonin receptors involved in impulsivity on the 5CSRT task. + identifies increases in premature responses, – indicates decreased impulsivity, =identifies the absence of any change. OFC= orbitofrontal cortex; BLA=basolateral amygdala; Nacc= Nucleus accumbens; mPFC= medial prefrontal cortex

Other studies using knock out animals have evaluated the role of the 5-HT1B receptor in impulsivity, and have suggested that the role of serotonin in impulse control is

site and receptor specific. The 5-HT1B receptor inhibits neurotransmitter release from both serotonergic and non-serotonergic neurons. In one study, 5-HT1B receptors were silenced in the whole brain, autoreceptors (located in terminal axons of serotonin neurons), or forebrain heteroreceptors (located on terminals of neurons from other neurotransmitter systems including glutamate, GABA, dopamine, and acetylcholine). Mice that lacked all 5-HT1B receptors were unable to withhold conditioned responses in impulsive action tasks (Differential-Reinforcement-of-Low-Rate responding and Go/No-Go). This impulsive phenotype was fully reversed by rescue of whole brain receptors expression in adulthood. Impulsivity was not affected by knockdown 5HT-1B autoreceptors. Finally, whole brain 5-HT1B knockdowns showed higher levels of dopamine in NAc, but not in dorsal striatum. Adult rescue of the 5-HT1BR normalized DA levels in the NAc, suggesting that this may be a mechanism by which these receptors affect impulsivity. Overall, the data suggest that serotonin can affect impulsive action through distinct circuits and during different time periods by acting through 5-HT1B receptors (Nautiyal, et al., 2015).

On a separate study, using knock-out 5-HT1B (5-HT1B KO) mice it was observed that these animals do not differ from wildtype (WT) in the acquisition of lever pressing response, reversal task or extinction of a previous learned response. However, 5-HT1B KO compared to WT showed higher response rate and lower number of reinforcements in a Differential-Reinforcement-of-Low-rate responding task, indicating enhanced impulsivity. On the other hand, 5-HT1A KO mice did not differ from WT in any of these tasks (Pattij, et al., 2003). Additionally, 5-HT1B KO mice do not differ from WT in a delay discounting task (Brunner & Hen, 1997), suggesting that the 5-HT1B receptors are involved specifically in impulsive action but not in impulsive choice.

Serotonin and impulsive choice

Finally, in some studies, serotonin depletion with 5,7-DHT does not appear to impact impulsive choice (Winstanley, et al., 2003; 2004). However, opposite effects of serotonin depletion have been observed by others, as animals infused with 5,7-DHT in dorsal and median raphe nuclei, showed and enhanced preference for smaller short delay reward, over a bigger and delayed one, supporting the idea that central 5-HT depletion increases impulsive choice (Bizot, Le Bihan, Puech, Hamon, & Thiébot, 1999; Mobini, et al., 2000a; Mobini, Chiang, Ho, Bradshaw, & Szabadi, 2000; Wogar, Bradshaw, & Szabadi, 1993). Additionally, systemic administration of selective serotonin reuptake inhibitors, Fluoxetine or Fluvoxamine, significantly increased the choice preference for large delayed reward, decreasing impulsive choice (Bizot, et al, 1999).

Different studies have pointed out the role of certain serotonin receptors in the control of impulsive choice, especially 5-HT1A and 5-HT3; however, their role is still not clear. Systemic administration of 5-HT1A agonist 8-OH-DPAT decreased impulsive choice in inherently high-aggression animals that also show impulsive choice, with no effects in low-aggression/not impulsive animals. On the other hand, administration of 5-HT3 antagonist Tropisetron decreased impulsive choice in impulsive high-aggression hamsters, as expressed by an increased preference for the big delay reward over the small immediate one. Low-aggression/not impulsive animals treated with Tropisetron decreased the preference for the delayed reward, evidencing increase impulsive choice. These results indicate a phenotypic-dependent reactivity to 5-HT1A and 5-HT3 receptors (Cervantes, Biggs, & Delville, 2010). Additionally, the impulsive–aggressive phenotype expressed by high-aggression hamsters has been associated with differences in 5-HT1A and 5-HT3

receptors, showing an increase in both, along with reduced 5-HT1A cells (Cervantes & Delville, 2009).

This role of 5-HT1 receptors has been supported by administration of Eltoprazine an agonist of 5-HT1A/B and partial agonist of 5-HT2C, which increased the choice of the large reward, decreasing impulsive choice. On the other hand, administration of GR-127935 a 5-HT1B antagonist had no effect on choice preference (van der Bergh, Bloemarts, Groenink, Olivier, & Oosting, 2006). Nevertheless, other studies have found the opposite. Specifically, systemic administration of 5-HT1A agonist 8-OH-DPAT or Buspirone increased the choice preference for small immediate rewards, showing increased impulsive choice (Winstanley, Theobald, Dalley, & Robbins, 2005; Bizot, et al, 1999; Liu, Wilkinson, & Robbins, 2004). This effect was blocked by previous administration of 5-HT1A receptor antagonist WAY 100635, which by itself had no effect on behavior. This indicates that the change in impulsivity observed following 8-OH-DPAT injections can be attributed to its action at 5-HT1A receptors (Winstanley, et al., 2005).

The role of other 5-HT family receptors on impulsive choice has shown that administration of Ketanserin a 5-HT2A antagonist, did not affect the choice preference in a delay discounting task. On the other hand, SER-082 a 5-HT2B/C antagonist significantly increases the preference for the large delayed reward, evidencing decrease impulsive choice. Finally, administration of SB-270146 a 5-HT6 antagonist did not affect the choice preference in a delay discounting task (Talpos, Wilkinson, & Robbins, 2006). This evidence suggests that 5-HT1A and possible 5-HT3, play an important role in the control of impulsive choice, however, their role is not clear yet.

I will be characterizing impulsivity in animals exposed to social stress in early adolescence, in order to propose neural systems associated impacted by this experience and associated with the behaviors observed.

EXPERIMENTAL OVERVIEW

The goal of this dissertation is to evaluate the long-term effects of chronic social stress exposure in early adolescence, and its relation to impulsive behaviors, to characterize the aggressive/impulsive profile observed in animals exposed to this experience. I will do it by answering the following questions:

1. *Is the enhanced aggression observed after early social stress exposure related to a lack of impulse control, especially the withholding of responses (action inhibition)?*

Impulsive action refers to deficits in the ability to inhibit behavioral responses. This failure in inhibition can be observed as failure to withhold, cancel or postpone a motor response. Previous research has shown that juvenile animals exposed to chronic social stress show enhanced aggression during adulthood, evidence by faster and higher number of attacks (Delville, et al., 1998; Wommack, et al., 2003). It is possible that this increased aggression is related to a lack of impulse control, especially the withholding of responses (action inhibition). Testing impulsive action (chapter 2) will provide a better characterization of the changes in behavioral responses after exposure to chronic social stress during adolescence associated with aggression and impulsivity in adulthood.

2. *Does exposure to chronic social stress in early adolescence have an effect on waiting impulsivity, another component of impulsive action?*

Impatience is another form of impulsive action referring to the inability to wait to produce a response in order to get a reward. As mentioned earlier, enhanced aggression exhibited by individuals previously stress in early adolescence, evidence by increase numbers of attacks and shorter latencies to do so, that may be associated with a lack of impulsive control. In this chapter (chapter 3), I will be testing whether this early experience

leads to impaired impulse control by addressing the waiting component of impulsive action.

3. *Are the effects of early social stress in impulsivity and aggression part of a more complex behavioral profile that include lack of perseverance?*

Perseverance refers to the ability to follow through with tasks from beginning to end in spite of difficulties, in other words it refers to the ability to not give-up easily. Altered perseverance is a component of the behavioral profile of some mental disorders associated with aggression, along with impulsivity (Hecht & Latzman, 2015). Impulsive individuals may cease prematurely to perform an under-rewarded task (decreased perseverance), or repeat actions for a desirable reward (increase perseverance), depending on testing conditions. Addressing perseverance in these studies (chapter 4) would lead to the development of a broad behavioral phenotype for animals exposed to stress in early adolescence.

The proposed studies will provide insight into the effects of chronic stress during adolescence, which is critical to the understanding of the impact of early trauma in the development of certain mental disorders associated with aggression and impulsivity such as borderline personality disorder, PTSD, anxiety, and depression among others.

METHODOLOGY

Animal care

Golden Hamsters were bred in the laboratory from a colony originally obtained from Harlan Sprague–Dawley (Indianapolis, IN) and were kept at the Animal Resource Center (University of Texas at Austin). Each litter of pups was culled to 4 males and 2 females at postnatal day 7 (P7) and housed with their mothers until P25. At P25 all animals were weaned and single-housed in Plexiglas cages (19 W x 43.2 D x 26.5 H cm) enriched with food piles and cotton pads. Weaning age is based on previous research in which social play within litters has been observed to peak at P21 and slows down dramatically afterwards, giving way to the posterior adult solitary life (unpublished data). Only males were kept for the present experiments based in previous research showing that early exposure to social stress in females does not affect the development of agonistic behavior (Taravosh-Lahn & Delville, 2004). Experimental hamsters were exposed daily to social stress from P28 to P42, while their controls were placed in empty clean cages. Training in conditioning chambers started between P50 and P70 and the animals were tested between P70 and P90 (Figure2).

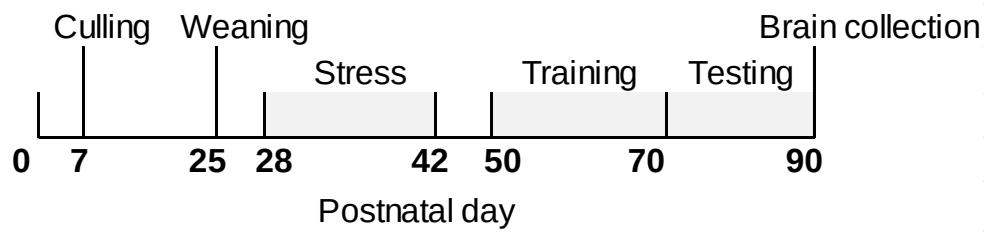


Figure 2. Timeline of behavioral procedures.

Animals were kept in a reverse light cycle (14/10 hr light/dark, lights off at 10:00 am) and received food and water ad libitum, except during the first one weeks of training. All behavioral procedures occurred during the first half of the dark cycle. Body weights were monitored three times per week during each experiment.

ADOLESCENCE

Adolescence is a developmental period characterized by numerous behavioral and neuroendocrine transitions. In male hamsters, testes grow from P30 to P60, and testosterone reaches a plateau around P60 (Wommack, et al., 2004; Vomachka & Greenwald, 1979). In addition, baseline cortisol levels rise starting around P28, and reach their peak around P70. As such, in hamsters, adolescence begins around P28, mid-adolescence occurs at P45, and P70 marks the beginning of adulthood (Wommack, et al., 2004). In my experiments, social stress starts at P28, which corresponds with early adolescence, and continues daily until P42, roughly mid-adolescence. Behavioral tests occur in adulthood (P70), which will reflect long term effects of social stress.

STRESS

Social stress (subjugation) was performed with a resident-intruder paradigm during the first half of the dark cycle, and under dim red light. Throughout early adolescence (P28-P42) hamsters were either exposed to a single-housed, larger, older and experienced resident male, or left alone in a clean new cage each day, for a period of 20 minutes in both cases. Resident-intruder dyads met up to twice across the fifteen days of stress.

Male hamsters are territorial and solitary animals (Rowell, 1961). In the laboratory, male hamsters readily attack smaller conspecifics placed in their home cage (Pellis & Pellis, 1988a; 1988b; Rowell, 1961). These agonistic encounters between juveniles and

adult male hamsters very rarely lead to injuries in the animals (Blanchard, Wall, & Blanchard, 2003). During these encounters juvenile animals investigate the adults (stretch attend postures), however over time this behavior is replace by avoidance of the adults (Lerwill & Makings, 1971).

The animals were observed during the encounters, and offensive responses by the residents like attacks and bites were recorded. Intruders were scored for submissive responses (tail up displays and on-back postures) and avoidance (running away from resident). Flank marks by residents were also scored. After each subjugation session, the intruders were checked for injuries and injured animals would have been excluded from the experiment. Attacks by adults on juveniles have never resulted in injuries over the past 15 years in our laboratory.

CONDITIONING CHAMBERS

Four individual conditioning chambers were used (30.5 W × 25.4 D × 30.5 H in cm, Coulbourn Instruments, Allentown, PA) controlled by Graphic State software, with aluminum sidewalls and ceiling, clear acrylic front and back walls and stainless-steel rod floors (rods 0.5 cm in diameter, spaced 1.0 cm apart). A wall-mounted magazine delivers banana food pellets (Dustless Precision Pellets®, 45mg, Primate Purified Diet, Banana flavor from Bio Serv) to a food cup mounted 2.5 cm above the floor and equipped with an infrared beam to detect nose-poking. Every chamber has one or two aluminum levers mounted at 2.5cm above the floor. Each conditioning chamber has a light (2-Watt white light) mounted 20 cm above the lever or at the opposite side of it, and a loudspeaker (8-ohm speakers) located at 25.5cm above the feeder. In the case of two levers present in the

chamber the light is located above the food cup dispenser. Each chamber is enclosed in a light and sound attenuated box (58.4 × 61 × 45.7 cm).

Every day hamsters were randomly assigned to a conditioning chamber, assuring that all animals experience the different possible positions of the lever and the light cue. Animals were kept in dark conditions throughout the transfer from the animal room to the testing room, which was illuminated with dim red light.

Conditioning procedure

At around P50 all hamsters began conditioning training. Animals were food restricted at 11:00pm the night before during the first 1 week of training as a motivational factor, and were tested between 11:00am and 3:00pm during the first half of the dark cycle. During food restriction animal's weight was closely monitored every other day to ensure no differences in body weight between groups and minimum weight losses (maximum 10% of body weight).

Magazine Training

Three sessions of 20 minutes each were carried out in order to allow the hamsters to make the association between the feeder and the conditioning chamber with a reward. During this phase, hamsters were food restricted the night before as a motivational factor. In these sessions, the food dispenser dropped a banana food pellet at random variable intervals of 60 seconds (VI60 seconds) until 20 pellets were delivered. After these three sessions all hamsters reliably retrieve the food pellets during the inter-trial-interval. Afterwards, all animals started their specific training protocol which differs between experiments.

CHAPTER 2: STRESS AND ACTION INHIBITION[1]

Experiment 2.1: Impulsive action in a Go-NoGo task

Previous studies have observed that exposure to stress during puberty impacts behavioral development in male golden hamsters. Social stress from exposure to aggressive adults in the first weeks of puberty accelerates the maturation of agonistic behavior, inhibits aspects of appetitive sexual behavior, and results in enhanced aggression in adulthood (Delville, et al., 1998; Wommack, et al., 2003; Bastida, et al., 2009). Similarly, social stress during development enhances aggression in other species, such as Rainbow trout, Nazca boobies, and Wistar rats (Øverli et al., 2004; Müller et al., 2011; Márquez et al., 2013).

Although the exact nature of this enhanced aggression in hamsters remains unclear, studies in rats suggest a more generalized effect impacting aspects of emotional reactivity and possibly impulsivity (Toledo-Rodriguez & Sandi, 2011; Márquez et al., 2013). These later observations are important as they suggest that exposure to stress during puberty alters neural circuits beyond those controlling social behaviors. For example, enhanced aggression manifested by elevated frequency of attacks and shorter latency to attack might reflect alterations in impulsivity and emotional reactivity (Haller, Harold, Sandi, & Neumann, 2014; Fodor et al., 2014; Coppens, et al., 2014).

In previous studies with adult hamsters, elevated attack frequency was correlated with impulsive choice, the preference for a smaller but immediate reward over a larger but delayed one (Cervantes & Delville, 2007; Cervantes & Delville, 2009). Perhaps enhanced

[1] This chapter is based on a previous publication: González-Martínez, L.F., D'Aigle, J., Lee, S.M., Lee, H.J., & Delville, Y. (2017) Social stress in early puberty has long-term impacts on impulsive action. *Behavioral Neuroscience*, 131(3), 249-261.
I (González-Martínez, L.F.) was in charge of experimental design, data collection, data analysis, discussion, and preparation of the manuscript. I also coordinated the two undergraduate students D'Aigle, J., and Lee, S.M. who significantly contributed to data collection and analysis. Lee, H.J. assisted in the design of the experiments and the discussion of the data. Delville, Y. supervised all aspects of the studies.

aggression observed in hamsters as a long-term consequence of exposure to stress during puberty might reflect their elevated impulsivity (i.e., they are having trouble inhibiting repeated attacks). However, impulsivity is not a unitary construct. As mentioned earlier there are at least two behavioral expressions of impulsivity: impulsive action and impulsive choice. Impulsive action refers to the inability to withhold a response and thereby reflecting poor response inhibition, whereas impulsive choice reflects a preference for suboptimal selections that include the preference for immediate reward over more beneficial but delayed ones (Brevers et al., 2012).

The present experiment evaluates the long-term effect of chronic exposure to social subjugation during early puberty on impulsive action in adulthood through a Go-NoGo task (Eagle, Bari, & Robbins, 2008; Cooper, et al., 2014). Perhaps enhanced aggression in animals exposed to social stress during puberty is caused by a lack of impulse control.

EXPERIMENTAL DESIGN

Two groups of animals were established: subjugated, n=15 and control, n=15; animals from the same litter were randomly assigned to either experimental or control group, and no more than 2 animals per litter were assigned to same group in order to avoid litter effects (Holson & Pearce, 1992). Animals were exposed to social subjugation and afterwards to the beginning of conditioning, as previously explained. After magazine training all animals were conditioned for 10 days to lever press in response to a light cue to receive food pellet rewards. Once proficient lever pressing was observed (more than 20 lever presses per session) food restriction stopped and Go signal training was performed for another 10 days. During this last phase of training, animals were rewarded with two food pellets if lever presses occur within 3 seconds of the light cue turning on and the lever

being extended. Promoting rapid responses is a key factor for Go-NoGo testing, therefore lever presses after 3 seconds were rewarded with only one food pellet. After P70 all animals were tested daily for two weeks on Go-NoGo sessions. During this phase, the same protocol for the Go signal training was used, except that 30% of trials were NoGo trials and a tone (4,500 Hz, 68dB) was presented simultaneously with the light cue.

During Go-NoGo sessions Go trials similar to the ones presented during Go signal training were used. In these trials, a light cue turned on, a lever was extended and lever presses within 3 seconds were rewarded with two food pellets, while lever presses over 3 seconds were rewarded with only one food pellet. These Go trials did not have a time limit and were randomly presented in addition to NoGo trials. The proportion of Go and NoGo trials was 70% and 30% respectively. During NoGo trials a light and a sound cue were presented at the same time that the lever was extended. Light and sound were programmed to last 15 seconds unless a lever press occurred. If animals press the lever during the presence of the light and sound, time restarted for another 15 seconds resetting the NoGo trial. If a NoGo trial was reset, 15 s needed to elapse without lever presses in order to receive one food pellet. However, if the NoGo trial was not reset at all, then at the end of the 15 seconds NoGo trial, two food pellets were delivered.

Go and NoGo trials were randomly alternated during testing sessions, however lever pressing during Go trials and absence of lever pressing during NoGo trials triggered the end of each trial and the beginning of the next one. Animals failing to inhibit lever pressing during NoGo trials kept resetting their NoGo trial. These animals could possibly get stuck on the same NoGo trial throughout most of a testing session. Alternatively, one animal may fail to respond to a Go trial, this keeping it going for most of a testing session. Because of these, I tested the animals several days during two weeks, and looked at

responses per NoGo cue presentation and per trial. One additional consequence of the experimental design is that each animal was likely be exposed to differing number of trials each session.

The following variables were recorded during this study during Go trials: number of Go trials completed, latency to lever press after cue presentation, and latency to retrieve the food after pressing during these trials. The following variables were recorded during NoGo trials: number of trials presented, number of cue presentations (as reset trials only ended after a successful cue presentation, this measure provides the number of trials and resets to each trial), total number of presses during trials (frequency of time resetting), number of trials without lever presses (successful NoGo trials), percentage of successful responses to cue presentations per session, and latency to lever press after cue presentation. These daily recordings were averaged for each animal (Cooper, et al., 2014) and the results compared between groups by Two sample t-test (two tailed). Additionally, the percentage of successful responses to NoGo cue presentations per session was analyzed with a two-way repeated measures ANOVAs [independent variables: groups (control or subjugated) and the successive test sessions over 13 days] followed by post-hoc tests (Tukey).

In addition, I looked at the frequency of sessions with either 50%, 75% and 100% successful NoGo trials across testing, and compared each percentage level between groups. In a manner inspired from psychometric detection functions (Parker & Newsome, 1998), the 50% level represents an absence of group difference, while the 100% level represents the maximum extent of group difference. I selected the 75% level as a midpoint detection threshold. Two samples t-test (two-tailed) were used to compare these frequencies at each percentage level as planned comparisons. Finally, a Chi-square test was used to compare the frequency of sessions with 100 or 0% successful NoGo trials.

I also looked at possible correlations between subjugation experience and impulsivity measures in experimental group. In particular, the total number of attacks received, number of tail-up displays and total number of on-back behavior recorded during subjugation was correlated with the following measures: total number of lever presses on Go trials, total number of lever presses on NoGo trials, number of NoGo cue presentations, percentage of successful responses to NoGo cue presentations, number of successful NoGo trials and frequency of days with 75% or more successful NoGo trials across the testing phase (Pearson for normally distributed measures, Spearman for non-normally distributed variables).

RESULTS

During training, animals in both groups lever pressed at the same rate throughout the sessions, showing an increase in lever presses and a reduction of the average time to lever press and to nose-poke to retrieve the food pellets towards the end of training. During the last 5 days of training, animals in both groups showed a similar rate of lever pressing (subjugated: 26.4±13.3, control: 22.3±15.0, M±SD). The percentages of lever presses occurring within 3 s of the light cue were similar between groups (subjugated: 35.0±20.4%, control: 24.0±17.7%). The latency to nose-poke after lever pressing was also similar between groups (subjugated: 11.2±9.0 s, control: 8.3±7.8 s).

During testing, the overall rate of lever pressing during Go sessions was reduced from the training period (subjugated: 15.2±8.0, control: 12.5±5.4), though the latency to nose-poke remained similar (subjugated: 9.1±6.9 s, control: 6.5±3.6 s). The data were analyzed separately for Go and NoGo trials during testing sessions. Subjugated animals were exposed to more Go trials than their controls [$t(24)=3.180$, $p<0.01$], though there was

no difference in their latency to lever press [$t(24)=1.255$, $p > 0.1$], or their latency to nose-poke after lever pressing [$t(24)=1.753$, $p>0.1$] (Figure 3A-3B).

NoGo trials produced different types of datasets: analyses of the daily data collected from the trials and cue presentations, as well as analyses on the relative success of the daily sessions. Previously subjugated animals were exposed to more NoGo trials [$t(24) = 3.376$, $p<0.01$], and more NoGo cue presentations [$t(24) = 3.644$, $p=0.01$] than their controls (Figure 3C-3D). Subjugated hamsters were also more likely and were faster to lever press [respectively, [$t(24) = 3.159$, $p<0.01$, $t(24) = -3.510$, $p<0.01$] (Figure 3E-3F). The analysis of success rates between groups varied between datasets. Over the repeated sessions, the rate of successful NoGo trials did not show group differences [($F(1,28) = 3,483$, $p = 0.072$], but a significant change over repeated sessions [$F(12,336) = 3,690$, $p<0.001$]. Post-hoc analysis showed that the first day of testing is significantly higher than days 4 ($p<0.05$), 6, 9, 12 ($p<0.001$) and 13 ($p<0.05$) (Figure 4A). There was no significant interaction between variables. On the other hand, while there was no group difference in the frequency of successful trials, subjugated animals were less successful than controls in the frequency of successful cue presentations [$t(24) = -2.702$, $p<0.05$] (Figure 4B).

In addition, NoGo data were also analyzed as a frequency of sessions presenting either 50%, 75% or 100% successful NoGo trials over the entire testing phase. The detection curves separated at the 75% threshold, as subjugated animals presented smaller frequencies (Figure 5A). The comparison of the frequency between groups was statistically significant for 75% [$t(28) = -2.207$, $p<0.05$] and 100% [$t(28) = -2.271$, $p<0.05$]. The relative distribution of failed and successful sessions (100% successful trials over time) was compared between groups. The analysis showed an overall greater frequency of

successful session and smaller frequency of failed session in control animals [$\chi 2 = 11.075$, $p<0.001$] (Figure 5B).

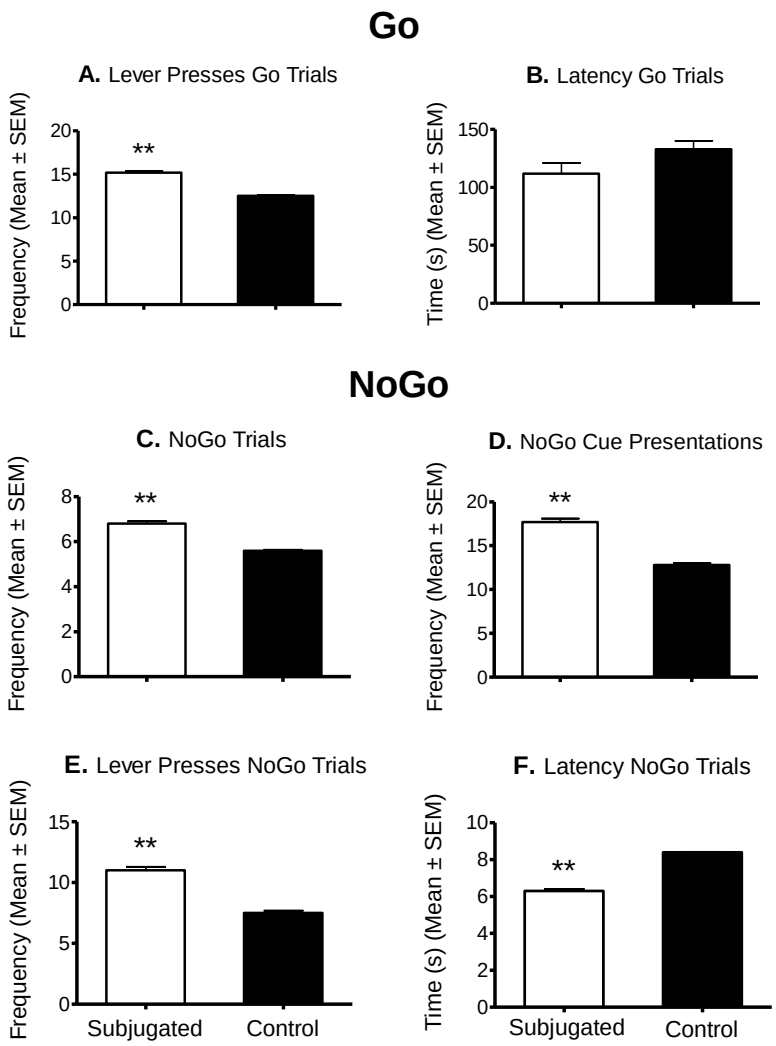


Figure 3. Results from Go–NoGo experiment. A: Lever presses go trials. Comparison of average lever pressing frequencies in response to a light cue (go trials). B: Latency go trials. Comparison of the average latencies to lever press in Go trials (there were no time limits to go trials). C: No go trials. Comparison of the average frequencies of no-go trials presented across testing. D: No go cues presentations. Comparison of the average frequencies of no-go cue presentations during testing. E: Lever presses no-go trials. Comparison of the average lever pressing frequencies during no-go trials. F: Latency no-go trials. Comparison of the average lever pressing latencies in response to cue presentation during no-go trials (no-go cue presentations had a 15-s time limit). (**) $p < .01$ (t tests).

Finally, I correlated measures recorded during the Go-NoGo testing phase with the behaviors observed during the subjugation period. While no variables were significantly correlated, some showed statistical trends. In particular, the frequency of on-back submissive postures was nearly correlated with number of successful NoGo trials across the testing phase (rs= 0.487, n=15, p=0.063). Similarly, the frequency of tail-ups displays was also almost significantly correlated with successful NoGo trials during testing (r=0.473, n=15, p=0.0749).

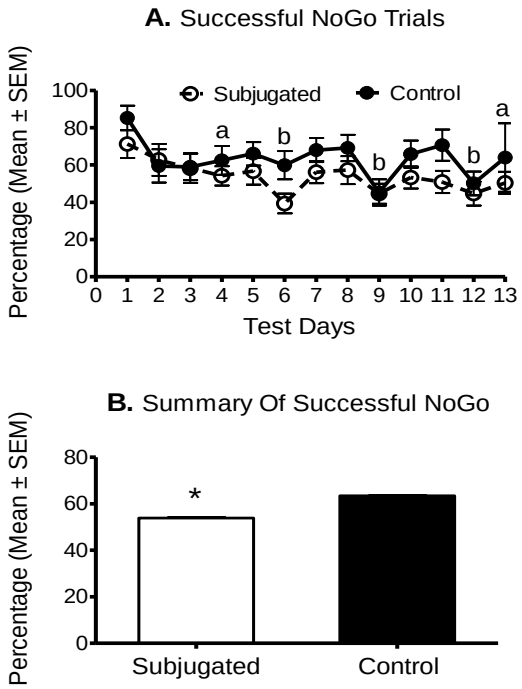


Figure 4. Results from Go–NoGo experiment. A: Successful no-go trials. Comparison of the daily successful rate of frequency of no-go cue (light tone) presentations across the testing phase between subjugated and control animals. B: Summary of successful no-go. Comparison of the average successful rate of no-go cue presentations across the testing phase between subjugated and control animals. (*) p< .05 planned group comparison. (a) p<.05 as compared to the first day of testing for the entire data set (Day 1). (b) p< .001 as compared to the first day of testing for the entire data set (Day 1).

DISCUSSION

The main goal of this study was to address whether exposure to social stress in early adolescence through repeated subjugations causes the long-term development of a pattern of impulsive action, in particular action inhibition. As these animals are known to be aggressive toward smaller individuals in adulthood (Wommack, et al., 2003), perhaps their frequent attacks on their opponents result from a lack in inhibitory control. The results obtained support this hypothesis. Hamsters were trained in adolescence and tested in adulthood over 30 days after the end of social stress in a Go-NoGo procedure addressing action inhibition (Eagle, et al., 2008; Cooper, et al., 2014). Overall, subjugated animals had fewer test sessions with successful NoGo trials.

During test trials, subjugated animals appeared more active as they were faster to lever press during NoGo trials and more likely to lever press, resetting the NoGo cues. Consequently, they were also exposed to more NoGo trials, and more NoGo cue presentations. Beside this greater frequency of NoGo trials, subjugated hamsters were also less likely to withhold lever pressing during NoGo cue presentations. These data are consistent with an effect of early stress enhancing action inhibition. This conclusion was supported by the analysis of the repeated sessions. Groups were compared at the 50%, 75% and 100% thresholds of successful trials per session over the testing phase. A separation by the 75% threshold indicates an early separation between the groups from the 50% level (Parker & Newsome, 1998). At the 100% threshold, over 30% of the sessions were successful in controls as compared to less than 20% in subjugated, confirming the data on successful NoGo presentations, and supporting the hypothesis of impaired action inhibition as a consequence of early social stress. It is worth noting that subjugated animals were also exposed to more Go trials, likely as a consequence of enhanced activity during NoGo trials.

In these Go trials, animals in both groups were remarkably slow to react to the light cue, though Go trials had not time limits as compared to the 15 second duration of NoGo cue presentations. This observation suggests that NoGo trials may have inhibited lever pressing responses to Go cue presentations, raising the possibility of a differential rate of extinction impacting this experiment. This possibility will be addressed later in the second experiment (Experiment 2.2).

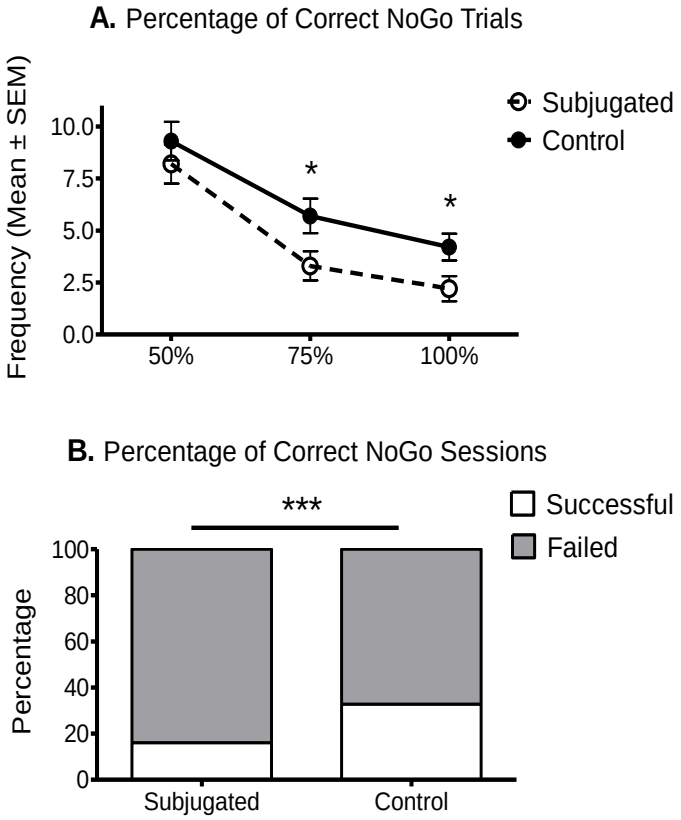


Figure 5. Results from Go–NoGo experiment. A: Percentage of correct no-go trials. Comparison of the frequency of sessions with 50%, 75% or 100% correct no-go trials across the testing phase. B: Percentage of correct no-go sessions. Comparison of the relative percentages of sessions per group where all no-go trials presented were either successful or failed no-go trials across testing phase. (*) $p < .05$ planned group comparison (t tests). (**) $p < .001$. Analysis of relative frequency distribution between groups (chi-square).

In previous studies, social stress in early adolescence has been associated with aspects of impulsivity. Rats exposed to social stress in early adolescence were viewed as risk takers and novelty seekers through open field tests containing a novel object or time spend in open areas of an elevated plus maze (Toledo-Rodriguez & Sandi, 2011). Nevertheless, the relation between stress in adolescence and impulsive action or action inhibition has not been addressed. As such, data from this experiment extend these previous observations and are the first to show an effect of social stress in early adolescence on impulsive action.

Finally, this experiment also included a correlation analysis between behaviors observed during subjugation and measures of impulsivity. First, there was absolutely no correlation between the frequency of attacks received and the measures of impulsivity. This observation may be interpreted as different from studies of abuse in humans. For instance, the intensity and frequency of exposure to bullying is predictive of symptoms of depression (Nansel, et al., 2001; Newman, et al., 2005). However, I found a nearly significant correlation with submissive behaviors such as on-back and tail-up postures. Animals most likely to perform these postures appeared somewhat less affected by repeated social subjugation. This observation is reminiscent of a similar correlation between submissive behavior during subjugation and the acceleration of the maturation of agonistic behavior by social stress during adolescence in hamsters (Wommack & Delville, 2003). Submissive responses to attacks could possibly be viewed as a form of stress coping in hamsters. In mice, resistance to attackers causes more detrimental effects of social stress (Mitra, Sundlass, Parker, Schatzberg, & Lyons, 2006). However, in rats, submissive behavior has been associated with more negative impacts of social stress (Stefanski, 1998).

Experiment 2.2: Extinction (Omission of the reward)

In the Go-NoGo task, the subject is required to inhibit a response in the presence of a discriminative stimulus (Izquierdo & Jentsch, 2012). However, during failed NoGo trials animals experience initially the absence of reward to a previously trained response. NoGo trials could be viewed as related to instrumental extinction (Izquierdo & Jentsch, 2012), as a loss of response after disruption of the association between two stimuli or between a response and a stimulus (Rescorla, 2001). Consequently, I considered necessary to test whether subjugated animals are resistant to extinction, as a control for the Go-NoGo experiment.

EXPERIMENTAL DESIGN

A group of 18 animals were distributed into subjugated and control groups (n=9 per gr) as explained above (Experiment. 2.1). After magazine training at P60, animals were conditioned for 10 days to lever press in response to a light cue to receive food pellet rewards. Once proficient lever pressing was observed food restriction stopped and training was carried over for another 10 days. Lever pressing during the light cue triggered the delivery of a reward and initiated an inter-trial interval (light cue off). Inter-trial duration was variable (5, 10 or 15 seconds randomly). The light cue was presented for a maximum of 30 seconds and absence of lever pressing during the light cue triggered an inter-trial interval.

Testing lasted from P80 to P94. During this phase, sessions were similar to training, but animals were no longer rewarded for lever pressing on cue. The duration of the light cue and lever presentation remained 30 seconds. The following measures were recorded during the extinction phase: average latency to lever press after cue presentation, frequency

of lever presses on cue, and frequency of lever presses on cue followed by a nose-poke to retrieve the food pellets (within 5 seconds). The latter measure was used as a control to verify that the light cue remained associated with lever pressing across the experiment. These data were compared between groups through two-way repeated measures ANOVAs [independent variables: groups (control or subjugated) and time including the average of the last 5 days of training and the repeated testing sessions] followed by post-hoc test (Bonferroni).

RESULTS

The results showed that both groups were equally capable of learning to lever press on cue in order to get food pellets reward. During training, animals in both groups lever pressed at the same rate throughout the sessions (Figure 6). They pressed the lever about 30 times per session in response to the light cue during the last 5 days of training. They also typically took about 10 seconds to lever press in response to the cue. During the testing phase, both groups maintained similar patterns of behavior. Lever pressing was reduced by the first testing sessions and further inhibited afterwards. In contrast, the latencies to lever press in response to the light cue became slightly longer.

The frequencies of lever pressing on cue decreased significantly over time from the average of the last 5 training sessions [$F(14,224) = 12.753$, $p<0.001$]. There was a significant reduction (ca. 30%) in both groups by the first day of testing ($p<0.001$). This reduction was further extended by another 30% by the last 6 days of testing ($p<0.001$) (Figure 6A). In addition, the frequency of lever pressing on cue immediately followed by a nose-poke was strongly reduced in both groups by the first day of testing and remained inhibited over repeated testing sessions [$F(14,224) = 26.785$, $p<0.001$]. This first day

reduction averaged 75% ($p<0.001$) (Figure 6B). By the end of the testing session, the animals barely had one lever press immediately followed by a nose-poke. In contrast, when they pressed the lever in response to the cue, the latency slightly increased over repeated testing sessions in both groups [$F(14,222) = 1.8086$, $p<0.05$], but it was only significantly higher on the 11 day of testing ($p<0.05$) (Figure 6C).

DISCUSSION

The results showed that both, subjugated animals and their controls, were equally capable of decrease lever pressing under an extinction paradigm. These data are consistent with the hypothesis that early stress has long-term impacts on action inhibition, supporting a condition-specific alteration in lever pressing responses after a change in rules of reward.

During this extinction experiment, the changes in the frequency of lever pressing on cue and the frequency of lever pressing on cue followed by a nose-poke presented similar downward curves. This observation means that the contingency between pressing the lever and going to the feeder lasted throughout the testing phase. What changed in this experiment was the response to the light cue. Consequently, the animals extinguished their conditioned response to the light. Interestingly, this extinction started by the first day of testing. Such a fast extinction was surprising as it was expected longer extinction periods (DiMeo & Wood, 2004; Dücker, Geyer, Schultze, & Stascheit, 1977), though previous studies in rats have reported similarly fast extinctions of operant tasks (Velley & Cardo, 1982). Perhaps, this fast extinction is a reflection of limited motivation to retrieve food pellets in this study, as hamsters were not food restricted during testing (Sturman, Mandell, & Moghaddam, 2010). This possibility would be consistent with the rather long (about 10 seconds) periods taken to nose-poke after lever pressing observed.

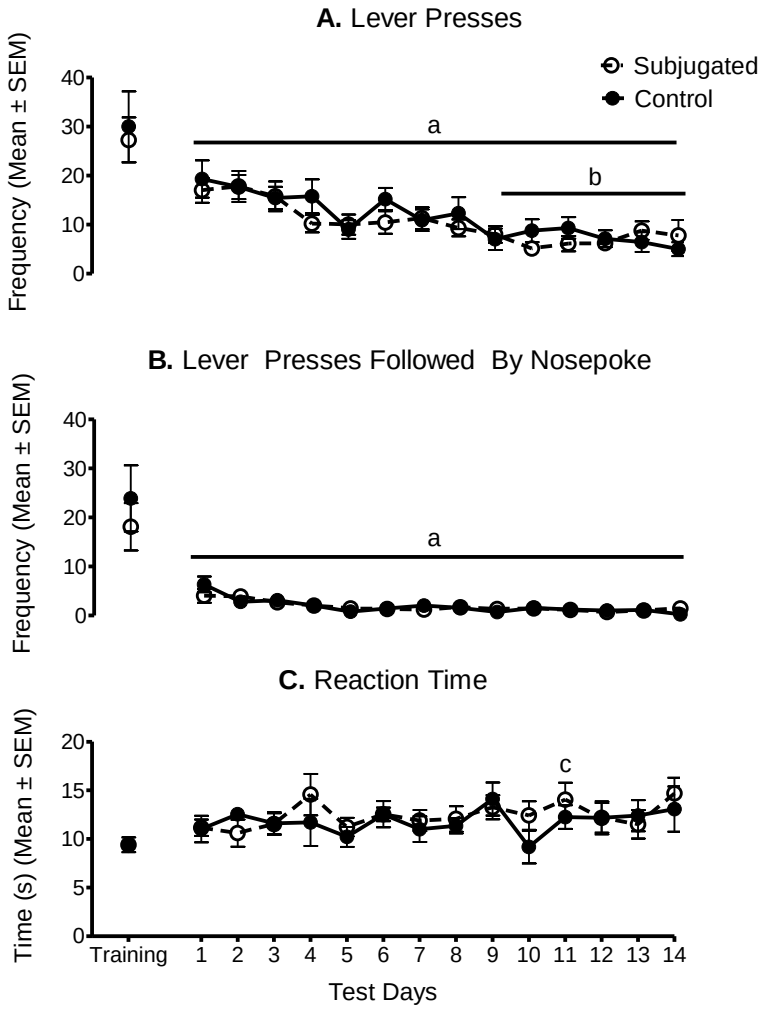


Figure 6. Results from extinction experiment. A: Lever presses. Comparison of lever pressing frequencies in response to the light cue over the extinction phase lasting 2 weeks and the average of the last 5 days of training. B: Lever presses followed by nose-poke. Comparison of lever pressing frequency in response to the light cue, and followed immediately by a nose-poke over the extinction phase and the average of the last five days of training. C: Reaction time (RT). Comparison of the RT to lever press in response to the light cue over the extinction phase and the average of the last 5 days of training. (a) $p < 0.001$, as compared to the average of last 5 days of training. (c) $p < 0.05$, as compared to the average of last 5 days of training. (b) $p < 0.001$, as compared to the first day of testing (Day 1)

The results obtained in this study are opposite to what has been observed in previous studies. Several studies have shown that exposure to stress disrupts extinction (Matsumoto, et al., 2008; Skelly, Chappell, Carter, & Weiner, 2015; Toledo-Rodriguez, Pitiot, Paus, & Sandi, 2012). Nevertheless, some of these studies have focused on different developmental periods, and used fear extinction to test the decrease in behavioral response. Testing the animals in fear extinction paradigms could be seen as an additional stressful challenge for the animals during testing. These methodological differences could explain the different results between the present experiment and previous studies.

The present study shows that juvenile stressed hamsters that show enhanced impulsive action in the Go-NoGo task do not have deficits in adjusting their behavior when the reward is fully omitted. However, in the Go-NoGo experiment the animals do not experiment a fully omission of the reward, therefore this could be seen as a too severe manipulation. As an alternative, a more appropriate control for the Go-NoGo experiment would be an experiment in which the reward is still delivered but delayed. This paradigm will be addressed in the next experiment (Experiment 2.3).

Experiment 2.3: Response to a non-trained and non-signaled delay

In the previous experiment the animals faced an omission of the reward, leading to extinction. Perhaps a less extreme situation in which animals can still receive rewards could lead to group differences as the ones observed in the Go-NoGo paradigm. For this, I decided to add a second control experiment for the possibility that lever pressing would still deliver a food reward, though after a substantial delay. In hamsters, the response to the introduction of a delay in reward has been correlated with individual differences in aggression (David, et al., 2004). Individuals identified as more aggressive maintained a higher rate of lever pressing. It is possible that a similar outcome would be observed in previously subjugated animals under these testing conditions.

EXPERIMENTAL DESIGN

A group of 18 animals were distributed into subjugated and control groups (n=9 animals per group) as explained above (Experiment. 2.1). On P60, after magazine training, the animals were trained to lever press for food pellet rewards. Conditioning chambers were equipped with 2 levers surrounding the food cup. Presses on the right lever were associated with an immediate delivery of two food pellets (active lever), while the lever on the left could also be pressed but was not associated with any reward (inactive lever). In these sessions, a light was not used, and no inter-trial interval was presented. The levers were continuously extended. Pressing the active lever turned on a light in the food cup as food rewards were delivered. That light turned off once the animal nose-poked to obtain the food reward. Once proficient lever pressing on the right lever was observed food restriction stopped, and training continue for another 10 days.

Testing sessions started around P80 and were repeated over 6 consecutive days. These test sessions were similar to training sessions, but pressing the active lever was associated with a food pellet reward delivered after a fixed 60 seconds delay, while the inactive lever remained unchanged. Pressing the active lever turned on the light in the food cup. That light remained on until the food pellet was delivered and the subject nose-poked afterward. During testing, the following variables were recorded: frequency of lever presses in the active and inactive lever, frequency of lever presses on the active lever followed by a nose-poke (within 5 seconds), ratio of rewards per lever presses [(number of food pellets/number of lever presses active lever)*100], and percentage of change as the frequency of lever pressing the active and inactive lever during each testing day over the average lever presses for the last 5 days of training.

Furthermore, the testing sessions were videotaped with a video camera located in the top of the chambers. The position of the animals inside the conditioning chambers was analyzed with these videos. During video reviews, the chambers was portioned into three areas: the back area (from the middle to the back end of the chamber), the area surrounding the active lever (from the middle to the front end of the chamber and from the right side wall to the middle of the food cup) and the area surrounding the inactive lever (from the middle to the front end of the chamber and from the left side wall to the middle of the food cup) (Figure 7). The time spent on each area during the entire 20 minutes session, as well as the first and last 10 minutes of the session was analyzed using EventCoder (1.0b10, generously donated by Dr. Michael Goldstein, Cornell University).

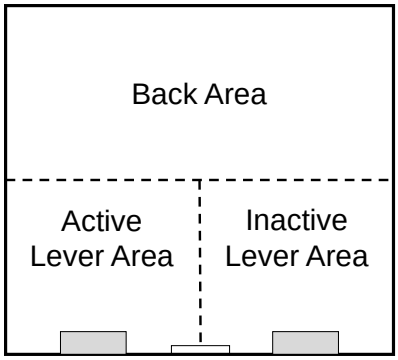


Figure 7. Schematic representation of conditioning chambers. Diagram representing active and inactive lever on each side of the frontal wall. Chambers were divided into active lever area, inactive lever area and back area of the chamber. Time spent on each area of the chamber was recorded and compared between groups.

Lever pressing during the testing sessions was analyzed through two-way repeated measures ANOVAs [independent variables: group (control or subjugated) and day of testing]. These analyses were followed by two sample t-tests or Man-Whitney test as planned comparisons for the first day of testing. Location preferences during the testing sessions were analyzed for the first day of testing through two-way repeated measures ANOVA [independent variables: group and area], followed by post-hoc tests (Tukey).

RESULTS

During training, the hamsters learned to press the active lever similarly in both groups, showing approximately a 15 to 1 preference for the active over the inactive lever. Lever pressing changed dramatically during testing in both groups (Figures 8 and 9), but some differences became apparent between groups. During testing, the frequency of pressing the active lever as well as the frequency to press this lever immediately followed by a nose-poke showed a significant reduction over repeated sessions [respectively, $F(5,80) = 5.368$, $p<0.001$; $F(5,80) = 11.656$, $p<0.001$], though there were no overall significant

group difference nor significant interactions between variables ($p>0.05$) (Figure 8A and 8B). This reduction was most pronounced in the control group, while subjugated animals showed only slight if any changes. Overall, the frequency of lever pressing the active lever and the frequency to press this lever immediately followed by a nose-poke was significantly reduced by the last days of testing as compared to the first day [respectively, $p<0.05$, $p<0.001$, Tukey]. Planned comparisons showed group differences on the first day of testing for both measures, as control animals lever pressed between 60 to 100% more than subjugated hamsters [respectively, $t(16) = -2.153$, $p<0.05$; $U = 64.500$, $p<0.05$]. Groups were similar on the following days of testing. In contrast to these data, the ratio of rewards obtained to lever pressing frequency increased gradually over testing, particularly in control animals [$F(5,79) = 2.929$, $p<0.05$] (Figure 8C). However, the post-hoc analysis only showed a statistical trend between the first and last days of testing ($p=0.05$, Tukey).

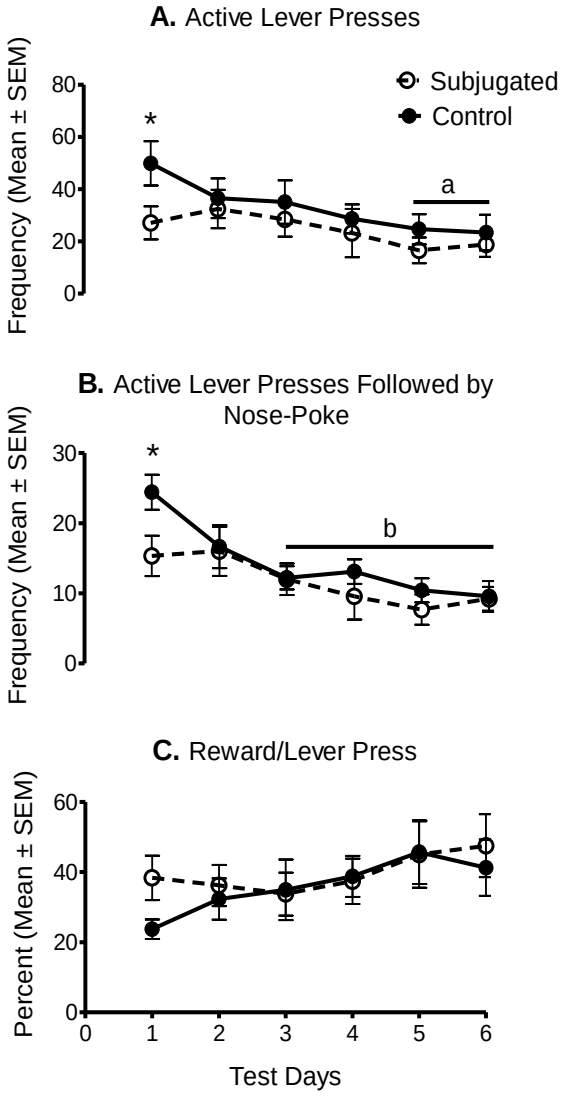


Figure 8. Results from delayed reward task (introduction of a 60-s delay in reward delivery after lever pressing). A: Active lever press. Comparison of lever pressing frequencies for the active lever between subjugated and control animals over the testing phase. B: Active lever presses followed by nose-poke. Comparison of lever pressing frequencies for the active lever followed immediately by a nose-poke between subjugated and control animals over the testing phase. C: Reward/lever press. Comparison of the ratio of rewards obtained per lever pressings between subjugated and control animals over the testing phase. (*) $p < .05$ planned group comparison. (a) $p < .05$ as compared to the first day of testing (Day 1). (b) $p < .001$ as compared to the first day of testing (Day1).

The data were further analyzed as percent change over training. Compared to the last 5 days of training, control animals showed a nearly 50% increase of lever pressing in the active lever on the first day of testing that decreased gradually over repeated tests to end with a ca. 20% reduction (Figure 9A). Subjugated animals maintained a ca. 50% reduction in lever pressing across the entire testing period. The overall changes over time in both groups were statistically significant [$F(5,80)=3.860$, $p<0.01$]. There was a combined reduction from the first day testing to the last two ($p<0.05$). However, overall group differences and the interaction between variables were not statistically significant ($p>0.05$). Planned comparisons showed a significant group difference on the first day of testing [$t(16) = 2.465$, $p<0.05$].

Lever pressing on the inactive lever was analyzed separately. The frequency of lever pressing the inactive lever remained low throughout the study (approximately 5 presses per session) in both groups. As compared to the last 5 days of training, there was an increase in the percentage of change in lever pressing which appeared to temper down over repeated sessions (Figure 9B). The overall reduction over time was statistically significant [$F(5,80) = 2.443$, $p<0.05$], as animals lever pressed less on the last day than the first ($p<0.05$). There was no difference between groups or significant interaction between variables ($p>0.05$).

As lever pressing appeared to differ between groups only on the first day of testing, the review of the videos collected during the experiment was focused on that day. Over this testing session, the animals were observed moving in all 3 sections of the testing chambers. Overall, animals spent the least amount of time by the inactive lever, and seemed to prefer the active lever area (Figure 10A). Indeed, the analysis of the entire test session showed significant differences between areas of the chambers [$F(2,32) = 14.665$, $p<0.001$],

as both groups of animals spent more time by the active lever and the back of the chamber than by the inactive lever ($p<0.05$). However, there was no significant difference between groups and no significant interaction between variables [respectively; $F(1,16) = 0.070$, $p>0.1$; $F(2,32) = 2.944$, $p=0.07$].

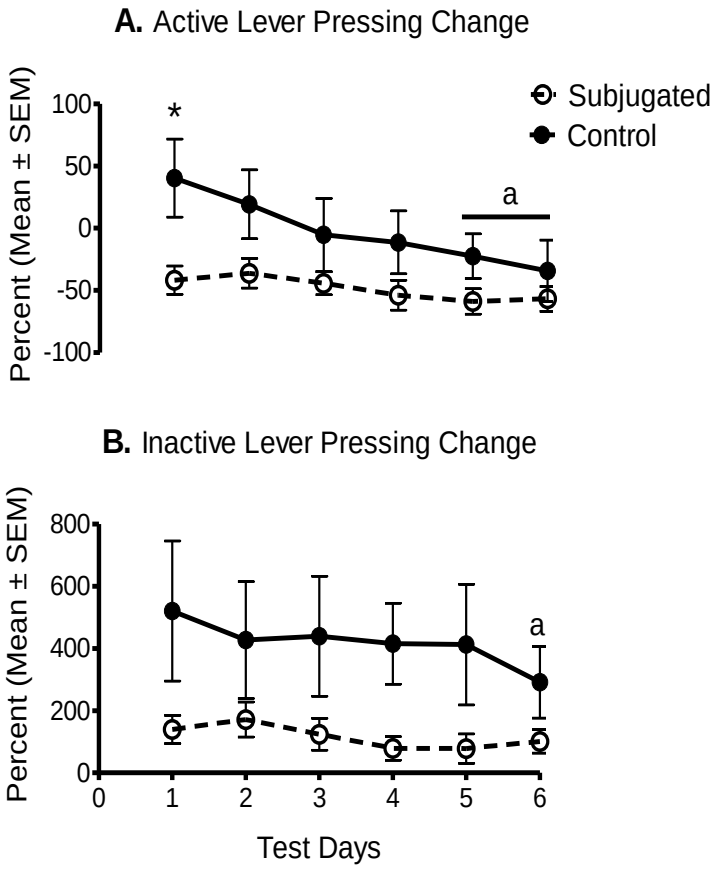


Figure 9. Results from delayed reward task (introduction of a 60-s delay in reward delivery after lever pressing). A: Active lever pressing change. Comparison of the percentage of change in pressing the active lever from the average of the last 5 days of training between subjugated and control animals over the testing phase. B: Inactive lever pressing change. Comparison of the percentage of change in pressing the inactive lever between subjugated and control animals over the testing phase. (*) $p< .05$ planned group comparison. (a) $p< .05$ as compared to the first day of testing (Day 1).

Interestingly, the preferences for the active lever area and the back of the chamber appeared to change over time, requiring separate analyses for the first and last 10 minutes of the test. The analysis of the time spent on each area during the first 10 minutes of the test showed different patterns of preferences between control and subjugated animals (Figure 10B). In the first half of the test, control hamsters had a strong preference for the active lever over the other two locations, spending nearly 50% of their time in that part of the chambers. In contrast, subjugated animals spent similar amounts of time by the active lever and the back of the chambers. This observation is supported by the analysis of the data. While there was no significant overall difference between groups [$F(1,16) = 0.447$, $p>0.1$], there was a significant differences between areas [$F(2,32) = 19.311$, $p<0.001$] and a significant interaction between group and area [$F(2,32) = 4.193$, $p<0.05$]. Subjugated animals spent more time than controls in the back of the chambers and less time by the active lever ($p<0.05$). Furthermore, control animals spent significantly more time in the active lever area than the other areas ($p<0.05$), while subjugated hamsters spent more time by the active lever than by the inactive lever ($p<0.05$).

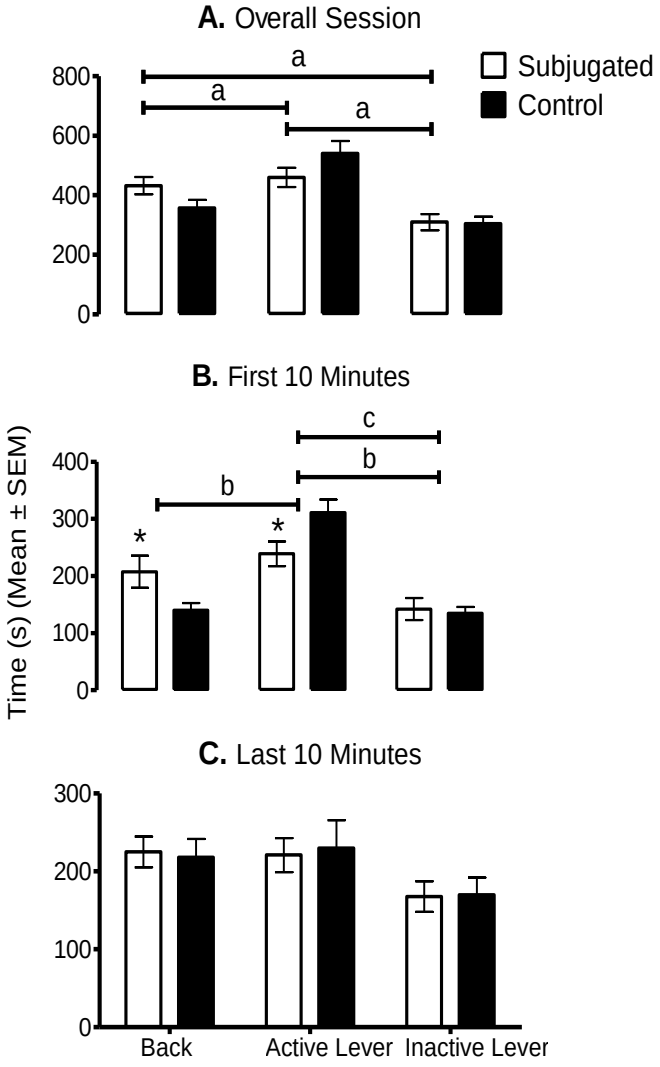


Figure 10. Results from delayed reward task (introduction of a 60-s delay in reward delivery after lever pressing). A: Overall session. Comparison of time spent on each of the three areas of the conditioning chambers during the overall 20-min session. B: First 10 min. Comparison of time spent on each of the three areas of the conditioning chambers during the first 10 min of the session. C: Last 10 min. Comparison of time spent on each of the three areas of the conditioning chambers during the last 10 min of the session. (*) $p < .05$ planned group comparison (t test). (a) $p < .05$ comparison between areas. (b) $p < .05$ comparison between areas for Control group. (c) $p > .05$ comparison between areas for subjugated group.

Finally, similar patterns of preferences became apparent between the groups in the second half of the test, as controls and subjugated spent similar amount of time by the active lever and the back of the chamber (Figure 10C). In fact, no significant differences were observed in any part of the analysis for that time period ($p>0.05$)

DISCUSSION

The results show that under a delay in reward subjugated animals were the first to inhibit their lever pressing. These data show an effect of early social stress on lever pressing activity under a long untrained and not signaled delay in the delivery of the reward. Furthermore, these data do not support a delayed extinction as a proposed alternative possibility for Experiment 2.1. Instead, the data indicate condition-specific alterations in lever pressing after changes in contingencies of the reward.

As expected from a previous study (David, et al., 2004), control hamsters responded first by increased lever pressing followed by a decrease over time. The pattern of subjugated animals was different: an immediate inhibition of lever pressing sustained over the rest of the study. These data are consistent with previous studies in which Roman high avoidance rats showed a significant decrease in lever pressing under a variable delay reward procedure (Coppens, et al., 2012), in a manner consistent with the faster inhibition of lever pressing in subjugated hamsters under a delay in reward in the present experiment.

In the context of this study, this rapid lever pressing inhibition conflicts with the higher frequency of failed NoGo trials observed in the first experiment. This observation further supports the specificity of the behavioral responses observed in each study under different changes in rules of reward. Animals exposed to social stress in early adolescence would be more likely to keep lever pressing when the chambers still deliver some food

pellets somewhat predictably, and would quit lever pressing immediately as the rewards are not coming fast enough or reliably enough. One possible interpretation of these differential changes in lever pressing after changes in rules of reinforcement may be drawn from Frustration Theory (Amsel, 1952; Amsel & Roussel, 1992). This theory predicts a higher rate of response to cues if rewards are distributed occasionally (Go-NoGo sessions), and a lower rate of responses if cues are no longer considered as reliable (60 seconds delay in reward). In the Go-NoGo experiment, the 3/1 ratio of Go to NoGo trials would have reinforced lever pressing in subjugated animals. In this experiment, the 60-second delay accelerated the loss of response.

On the other hand, it is interesting that the results of this third experiment conflict with those of a previous study in adult hamsters (David, et al., 2004). In that previous study, animals characterized as aggressive maintained a high rate of lever pressing under such a long delay in reward. These animals were later found impulsive under a delay-discounting paradigm (Cervantes & Delville, 2009). The effects of social stress in early adolescence also lead to elevated aggression in adult hamsters (Wommack, et al., 2003), but in this case these animals respond differently to a delay in reward. These data suggest that the behavioral correlates of aggression may vary greatly between manipulations made to the animals or the type of animals. For instance, Roman high avoidance rats are more impulsive than low avoidance individuals, while being less aggressive (Coppens, de Boer, Steimer, & Koolhaas, 2013; Moreno, et al., 2010). In contrast, testosterone-treated rats are also aggressive, but not necessarily impulsive (Cooper, et al., 2014; Wallin, et al., 2015; Wood, et al., 2013). Perhaps there are different types of offensive aggression each differentially correlated with different forms of impulsivity, and different responses to a sudden delay in reward.

In this study, changes in lever pressing were confirmed by review of videos collected during testing. During the progress of the first testing session, as the animals stopped lever pressing, they also changed their location preferences. In particular, subjugated animals were the first to lose their preference for the area near the active lever. This rapid inhibition may reflect a form of impatience in these animals or possibly a lack of perseverance. This issue will be tested in the next studies (Chapters 3 and 4), as it may be a key characteristic of the behavioral profile related to stress exposure in adolescence.

CHAPTER 3: STRESS AND PATIENCE

Experiment 3.1: Waiting to respond in a modified 5-Choice-Serial-Reaction-Time task

In previous studies, juvenile hamsters exposed to chronic social stress displayed enhanced offensive aggression (Wommack, et al., 2003) and enhanced impulsive action during adulthood (Experiment 2.1). As mentioned above, impulsive action has two major components: withholding (action inhibition) and postponing (waiting). The effect of stress on action inhibition (the ability to inhibit a response) has been tested (chapter 2). Because previous studies in our lab have shown that subjugated animals were faster to attack as adults, in addition to attack more (Delville, et al., 1998), maybe this reflects an inability to delay actions and could be explain as impaired waiting impulsivity. Perhaps the two forms of impulsive action can be linked to describe the phenotype of these animals. Therefore, it is important to evaluate if the exposure to chronic social stress during adolescence is also associated with the other component of impulsive action, waiting impulsivity, which refers to the tendency to respond before a target onset. In this chapter, I tested impulse control as the ability to wait to perform the conditioned behavioral response by evaluating premature responses in a modified 5-Choice-serial-reaction-time task (5-CSRT).

EXPERIMENTAL DESIGN

A group of 29 animals was distributed into subjugated and control groups (subjugated: n=15; control: n=14) as explained above (Experiment 2.1). All hamsters began conditioning training between P50 and P60, and were food restricted during the first 10 days of training as a motivational factor.

Conditioning chambers

Eight 5-Choice-serial-reaction-time chambers (Figure 11) were used to test stress and patience. The chambers (25x25x25 cm3) consist of two clear plastic walls, and two aluminum walls, one of which is curved and equipped with 5 circular holes of 2.5 cm sides, 4 cm deep, and positioned 2 cm above the grid floor. The opposite wall is not curved and is equipped with a food well (5x5 cm2) connected to a pellet dispenser. All the circular holes and the food tray have infrared beams in order to detect the animal's responses. The food tray and each hole are illuminated independently according to the task contingencies. The chambers have a house light (3 W) that illuminates the box during the task. The floor is a metallic grid and the roof is made of metal. Each chamber is enclosed in a light and sound attenuated box (58.4 × 61 × 45.7 cm).

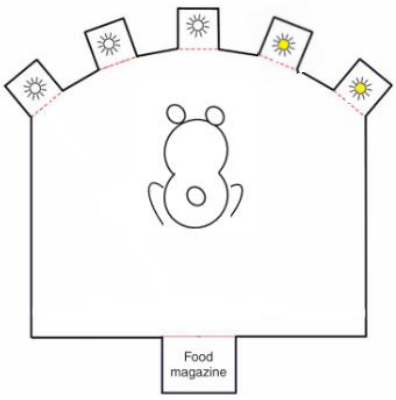


Figure 11. Schematic representation of a conditioning chambers used to test stress and waiting (adapted from Bari, Dalley, & Robbins, 2008).

Three sessions of magazine training were carried out so the hamsters could associate the feeder and the conditioning chamber with getting a reward. In these sessions, the food dispenser dropped a food pellet at random variable intervals of 60 seconds (VI-60

seconds) until 30 pellets were delivered. After these sessions, all hamsters reliably retrieved the food pellets within the 10 seconds after pellet delivery.

After magazine training, all hamsters were trained for 7 days to nose-poke in the ports to receive food pellet rewards. On each trial, a main house light was illuminated and 2 seconds later all five ports were illuminated for 30 seconds. If a nose-poke in any port was detected, the light in the ports and house light turned off, while the light in the food cup turned on as a food pellet was delivered. This light remained on for up to 5 seconds afterwards. During that time, nose-pokes in the food-cup triggered a 3-second inter-trial interval (all lights off). All lights were turned off for 3 secs if the animal did not get into the food cup during this 5-sec interval. Nose-pokes in the food cup within 5 seconds post-food delivery were recorded as a measure of learning. Nose-pokes performed during the 2 secs-interval after the house light turned on and before the illuminations of the ports did not have any consequence. After 7 days, food restriction stopped and training continued for another week with all five ports illuminated. Once hamsters met a criterion of 65% or more responses per session, training continued with the illumination of only four ports randomly located. Nose-pokes in the non-illuminated port did not have any consequence. Once animals meet criterion of 65% correct responses, the number of ports illuminated was reduced to three randomly located ports at a time, and later to only two adjacent randomly located ports at a time.

After nose-poking training, animals were tested for 10 days in a similar procedure to the last five days of training. After the illumination of the chamber with the house light turning on, only two adjacent and randomly located ports were illuminated, however the duration of the waiting period, i.e. the time between main house-light turning on and illumination of the ports, was variable (2, 5, 10, 20, 40 seconds) (Figure 12). These delays

were randomly presented during each session. Nose-pokes during these delay periods did not result in food pellet delivery (and did not trigger a time-out period), therefore responses during this period were recorded but did not have any consequence. I decided not to punish premature responses with a time-out because this would add an extra variable during testing. Because the main goal of this study was to evaluate if there was a difference between groups in how they behave when they have to wait to respond, I consider that the most appropriate testing protocol should record premature responses but not associate any consequence with these responses. These tests sessions lasted for 30 minutes.

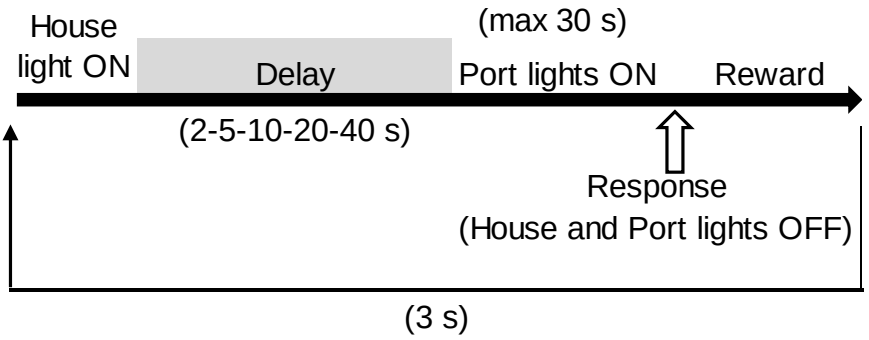


Figure 12. Timeline of a testing trial in the task for waiting impulsivity. Each trial begins with all lights off. The house light turns on, and after a varying random delay of 2, 5, 10, 20 or 40 seconds, the light in two random adjacent ports turns on and remains on for a maximum of 30 seconds. If a nose-poke is detected in any of the ports illuminated, the house light and the ports light turn off, and a light in the food cup turns on as a food pellet reward is delivered. After 5 seconds, or a nose-poke in the food cup, the light in the food cup turns off. All lights in the conditioning chamber remain off for 3 seconds before a new trial begins. Delays were presented in a random order each testing sessions, during 5 days of testing.

The following parameters were assessed during this study per each delay presented: accuracy, which reflects the nose-pokes performed in the ports that were illuminated [(correct responses/correct+incorrect responses)*100]; percentage of errors, which

correspond to the nose-pokes performed in ports that were not illuminated when others were [(incorrect responses/incorrect+correct responses)*100]; omissions, reflecting trials in which animals did not perform any nose-poke when the ports were illuminated [(omitted/trials presented)*100], and latency to perform a correct nose-poke. The monitoring of accuracy was included to determine whether the testing procedure does not impair the learned response. Additionally, premature responses were recorded as a measure of impulsivity, and reflect the responses made before the light in the ports turned on (i.e. during the delay period). These premature responses were calculated per delay as frequency of nose-pokes during the delay period, and as a percentage of the sum of all the responses [(premature responses per delay/∑premature+∑correct+∑incorrect responses)*100].

These daily recordings were averaged for each animal and the results compared between groups with two-way repeated measures ANOVA (independent variables: group and delay). These analyses were followed by post-hoc tests (Tukey).

Additionally, separate linear regression analyses were performed for each group with delay as explanatory variable and percentage of premature responses as outcome variable. Afterwards, the slopes obtained from the regression analysis were compared between groups. This analysis was performed via dummy-regression model (Fox, 2016, pp.122), with the dummy variable representing the variable group.

RESULTS

During training, animals in both groups learned to nose-poke throughout the sessions. There was no consistent group difference across training sessions. During the last five days of training, animals in both groups showed a similar rate of nose-poking (subjugated: 26.1 ± 4.4, control: 29.2 ± 4.4, mean ± SD). Likewise, accuracy was similar

between groups (subjugated: 85.5 ± 0.9%, control: 89.4 ±1.2%), as well as percentage of errors (subjugated: 14.5 ± 0.9%, control: 10.6 ± 1.2%) and omissions (subjugated: 58.3 ± 2.0%, control: 56.2 ± 1.6%). The latency to nose-poke after the light in the ports turned on was also similar between groups (subjugated: 12.82 ± 0.31 seconds, control: 12.91 ± 1.39 seconds). Finally, the latency to nose-poke in the food cup to retrieve the reward within 5 seconds after food delivery, was similar between groups (subjugated: 3.07 ± 0.05 seconds, control: 3.12 ± 0.08).

During testing, animals in both groups maintained their accuracy, rate of errors, omissions, and latency to respond regardless of the delay presented. There was no significant difference in accuracy between groups [$F(1,108)= 0.221$, $p>0.05$], both groups were similarly accurate regardless of delay presented [$F(4,108)= 0.891$, $p>0.05$], and there was no interaction between group and delay for this measure [$F(4,108)= 1.418$, $p>0.05$] (Figure 13A). Both groups also presented similarly low error rates [$F(1,108)= 0.221$, $p>0.05$], which did not change with increasing delays [$F(4,108)= 0.891$, $p>0.05$]. Additionally, the interaction between groups and delay was not statistically significant [$F(4,108)= 1.418$, $p>0.05$] (Figure 13B). Finally, there was no difference between groups in the percentage of omissions [$F(1,108)= 0.021$, $p>0.05$], regardless of the delay presented [$F(4,108)= 0.261$, $p>0.05$]. Furthermore, there was no interaction between group and delay [$F(4,108)=1.023$, $p>0,05$] (Figure 13C).

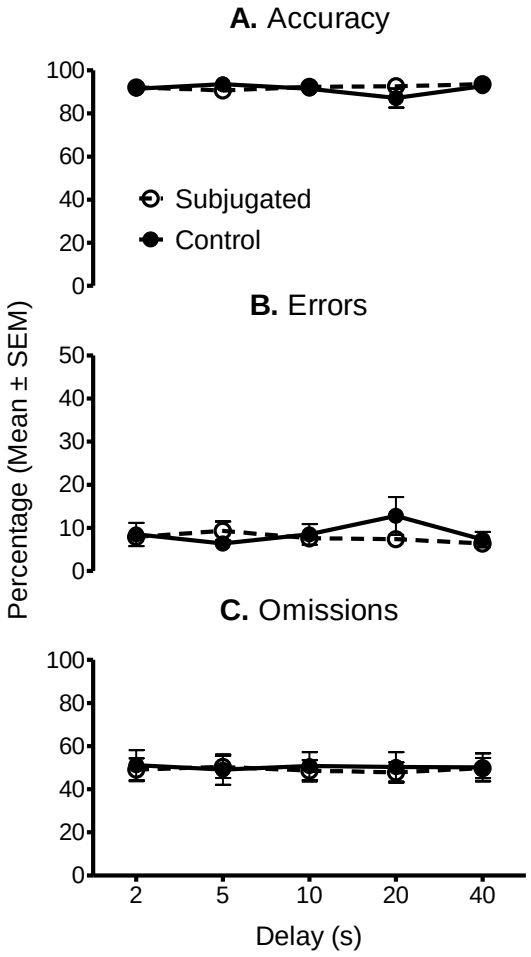


Figure 13. Results during testing in the task for waiting impulsivity. A: Accuracy. Comparison of the average percentage of nose-pokes in any of the two, adjacent illuminated ports (ports were illuminated for 20 seconds). B: Percentage of errors. Comparison of the average percentage of nose-pokes in non-illuminated ports when others where. C: Percentage of omissions. Comparison of the average percentage of trials in which nose-pokes were not detected in the ports during the 20 seconds respond period.

In this task, the measure of impulsivity consisted of premature responses during the wait period (i.e. responses made before the light in the ports turned on). The frequency of these responses grew gradually in both groups with longer delays. However, this increase

appeared sharper in control animals, leading to higher frequencies by the 40 seconds delay. The analysis showed that there was no differences between groups [$F(1,108)= 2.80$, $p>0.05$], but there were significant differences between delays [$F(4,108)= 64.40$, $p<0.01$], and a significant interaction between group and delay [$F(4,108)= 2.85$, $p<0.05$]. The frequency of premature responses was significantly higher by 10, 20, and 40 seconds delay ($p<0.001$), as compared to 2 and 5 seconds. Furthermore, animals in control group had significantly higher frequency of premature responses by the 40 s delay compared to subjugated animals ($p<0.01$) (Figure 14A). When looking at the percentage of premature responses as an indicator of the proportion of all responses that were premature, the same is observed. The percentage of premature responses grew gradually in both groups with longer delays. Though this increase appeared sharper in control animals, leading to higher rates by the 40 seconds delay. The analysis showed that there was no difference between groups [$F(1,108)= 3.191$, $p>0.05$], but there were significant differences between delays [$F(4,108)= 210.12$, $p<0.001$], and a significant interaction between group and delay [$F(4,108)= 5.462$, $p<0.001$]. The rate of premature responses was significantly higher by 10, 20, and 40 seconds delay ($p<0.001$), as compared to 2 and 5 seconds. Furthermore, animals in the control group had a significantly higher percentage of premature responses in the longest 40 seconds delay ($p<0.001$) compared to subjugated animals (Figure 14B).

There was no difference between the groups in the latency to perform a correct nose-poke [$F(1,108)= 0.018$, $p>0.05$], but there was an overall difference between delays [$F(4,180)= 2.612$, $p<0.05$], and no significant interaction between group and delay [$F(4,180)= 0.663$, $p>0.05$]. However, the post hoc analysis did not show any significant difference between delays. (Figure 14C).

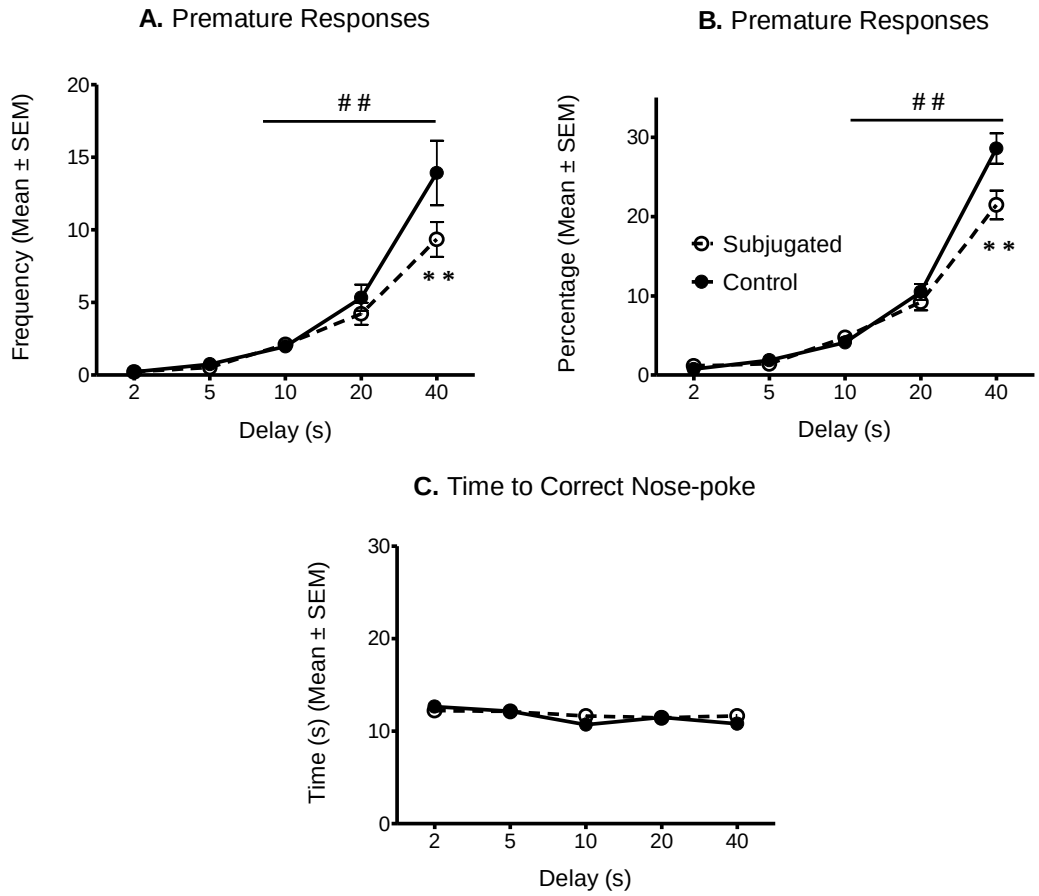


Figure 14. Results during testing in the task for waiting impulsivity. A: Frequency of premature responses during testing. Comparison of the average frequency of nose-pokes in any port before the target ports were illuminated. B: Percentage of premature responses during testing. Comparison of the average percentage of nose-pokes in any port before the target ports were illuminated. C: Latency to perform a correct response. Comparison of the average nose-poke latency in response to illumination of two adjacent ports. (**) <0.001 between groups. (##) < 0.001 with 2 and 5 second delay.

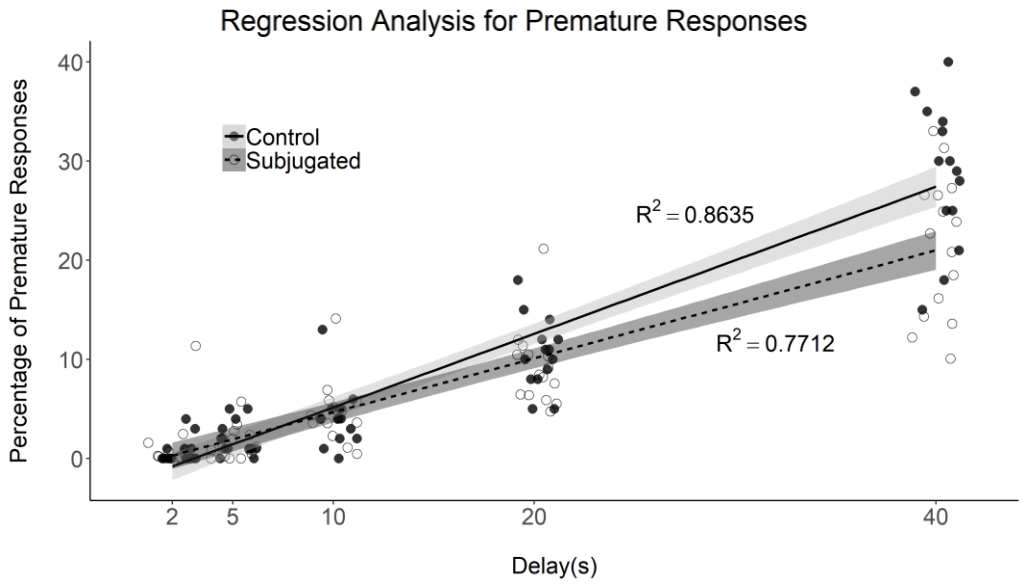


Figure 15. Figure showing the linear regression analysis for premature responses in the task for waiting impulsivity. The outcome variable was percentage of premature responses, and the predictor variable was delay. Closed circles: control animals, open circles: subjugated. Solid line: regression line for control group, dashed line: regression line for subjugated group. Gray areas represent the confidence intervals of the regression lines.

Finally, the results of the regression analysis for the control group indicated a significant relation between delay and percentage of premature responses, $\beta = 0.7420$, $t(68) = 20.739$, $p<0.001$, 95% CI [0.67, 0.81]. Delay also explained a significant proportion of the variance in the percentage of premature response, $R^2 = 0.8635$, [$F(1,68) = 430.09$, $p<0.001$]. For the subjugated animals, delay was also significantly related to the percentage of premature responses, $\beta = 0.5451$, $t(73) = 15.686$, $p<0.001$, 95% CI [0.47, 0.61]. Delay also explained a significant proportion of the variance in the percentage of premature responses, $R^2 = 0.7712$, [$F(1,73) = 246.04$, $p<0.001$] (Figure 15). Afterwards, we added a comparison of the regression slopes between the groups with a dummy-variable. The

comparison was statistically significant [t(141)= -3.945, p<0.01], showing that the slope in the control group was significantly higher than in the subjugated animals (Figure 16).

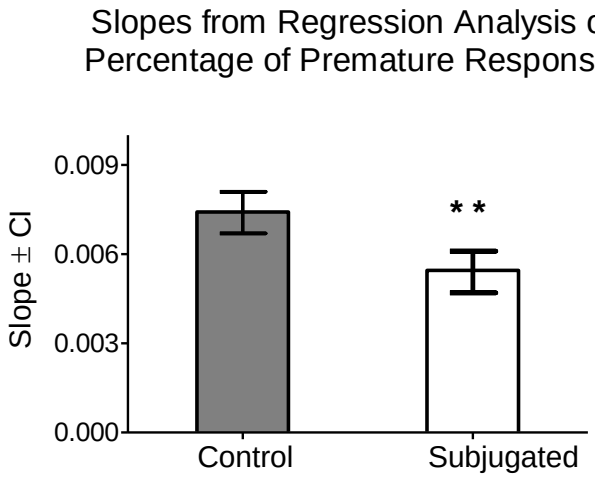


Figure 16. Comparison of the slopes obtained in the linear regression analysis. Representation of the slopes and 95% confidence intervals obtained in the regression analysis with percentage of premature responses as outcome variable, and delay as predictor variable. (**) <0.01 between groups.

DISCUSSION

The main goal of this experiment was to evaluate whether exposure to social stress in early puberty causes long-term deficits in impulsive action, in particular the capacity to postpone a response (waiting impulsivity). This possibility was tested using a modified 5-CSRTT procedure adapted to hamsters. In the study, the key measure of impulsive action consisted of the frequency of premature responses under lengthening delays. There was a significant difference between groups by the longest delay (40 seconds) with animals previously exposed to social stress presenting lower rates of premature responses. In addition, a regression analysis of the premature responses over delays was included. This analysis pointed to a significantly sharper slope in control animals, supporting the group

differences in premature responses. Consequently, exposure to stress during early puberty does not impair the capacity of an individual to delay an action. Instead, it enhances the capacity to delay responses. This observation contrasts with earlier results report on action inhibition (Chapter 2, Experiment 2.1) which was impaired in animals exposed to stress in early puberty. Thus, early stress has opposite effects on the components of impulsive action, reducing the capacity of an individual to inhibit a response while enhancing its capacity to wait before initiating it. To my knowledge, such findings have never been reported in animal models of stress and impulsivity.

This study included observations of accuracy, error rates and omissions. These measures did not change with increasing delays and did not differ between groups. Thus, the effects of exposure to social stress in early adolescence are selective to premature responses in this testing procedure. Although the omission rate is around 50% and could be considered high, this is an indicator of motivation rather than learning. Considering that the animals were not food restricted during testing, it is possible that they were not highly motivated to respond for the food pellet reward and did not respond in half of the trials presented. However, considering the accuracy rate, which is above 80%, this indicates that the animals learned the task and responded in the correct ports that were illuminated in those trials that they did respond.

While this study is the first to address waiting impulsivity and social stress in puberty using a modified 5-CSRTT procedure, this task has been previously applied to other models of stress. First, a 5-CSRTT procedure was used in Wistar rats exposed to stress in early puberty (Tzanoulinou et al., 2016). In that study, stress consisted of predator odor and exposure to an elevated platform. However, their 5-CSRTT procedure was optimized for testing attention instead of waiting impulsivity. Second, a 5-CSRTT

procedure addressed waiting impulsivity in rats exposed to stress in infancy and/or early puberty (Baarnedse et al., 2013). In that study, stress consisted of social isolation and experimental animals showed increased rates of premature responses. While this outcome is the opposite of the present findings, it is important to note that social isolation and social subjugation also have opposite impacts on social behavior as well. In rats, social isolation in infancy and/or early puberty enhances later play fighting activity between individuals (Varlinskaya, Spear, & Spear, 1999). In hamsters, social subjugation in early puberty does not enhance play fighting, but it accelerates its maturation into aggression (Wommack et al., 2003). These different stressors are likely associated with different neuroendocrine consequences that could explain different behavioral outcome. For instance, social subjugation in adolescence reduces baseline testosterone levels in hamsters (Wommack et al., 2004), while social isolation does not (Amstislavskaya, Bulygina, Tikhonova, & Maslova, 2013).

Previous studies have related aggression with impulsivity. Hamsters exposed to stress in early puberty, become more aggressive as adults (Wommack et al., 2003), but show opposite associations with action inhibition (Chapter 2, Experiment 2.1) and waiting impulsivity (this experiment). Thus, the types of impulsivity associated with aggression differ between models. Hamsters selected for repeated aggressive responses, also show intolerance to delays in delay discounting procedures (Cervantes & Delville, 2009). In rats, exposure to testosterone enhances aggression but does not impact action inhibition (Cooper et al., 2015). However, testosterone treatment impacts impulsive choice, enhancing tolerance to delays in rewards, increased effort, and hazards, while impairing tolerance to decreased odds of rewards (Wallin-Miller, Li, Kelishani, & Wood, 2018; Wallin et al., 2015; Cooper et al., 2014; Wood et al., 2013). Furthermore, associations have also been

made between impulsive action tested in the 5-CSRTT procedure and aggression, using Roman High and Low avoidance breeds of rats. Adult Roman Low Avoidance animals are more aggressive (Coppens et al., 2012), and like our stressed hamsters are more tolerant to delays to initiate an action, while also being more tolerant to delayed gratification in the delay discounting task (Moreno et al., 2010). However, after social stress in puberty, Roman High Avoidance animals become more aggressive and show a lack of tolerance to delays in reward (Coppens et al., 2012). Thus, aggression defined as higher rate of offensive responses during agonistic encounters can be associated with different forms of impulsivity.

Experiment 3.2: Waiting to receive a reward in Variable delay in delivery of reward (VDDR) task

In the task testing waiting impulsivity, the subject is required to wait to respond in the presence of a discriminative stimulus (Bari et al., 2008). However, my previous studies have shown that when animals encounter a 60 seconds delay in the delivery of the reward, they rapidly suppress their responses (Experiment 1.3) suggesting a sensitivity to delayed gratification. Therefore, I determined it was necessary as a control for the specificity of the effects caused by the timing of the delay, to present the delay in a different moment of the procedure and test whether subjugated animals were affected in their capacity to wait to receive a reward, (i.e. waiting to respond vs. waiting for a reward).

EXPERIMENTAL DESIGN

A separate set of animals (subjugated: n=10; controls: n=10) was trained in the same protocol as described for experiment 3.1, and tested in a similar protocol, except that the delay was introduced after the response and corresponded to a delay in the delivery of the reward (Figure 17). Once proficient nose-poking in the illuminated ports was observed, animals were tested for 5 days under varying delays in the delivery of the reward after correct nose-poke responses. Animals were tested each day to a unique delay of 0, 10, 20, 40 or 60 seconds in the delivery of the reward. The delay for each day of testing was randomly selected, controlling the possible effect of testing in an increasing or decreasing order. Due to previous evidence in which the absence of an immediate reward lead to a rapid extinction (Chapter 2, Experiment 2.2), animals were exposed to 3 consecutive days of training after each test session. Pilot studies showed that three days of re-training were sufficient.

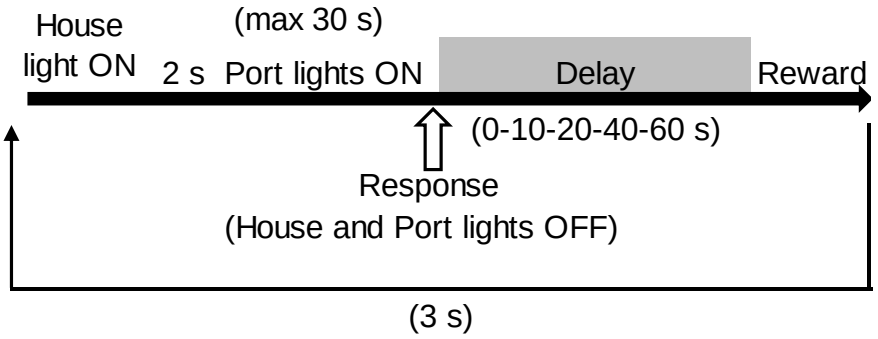


Figure 17. Timeline of a testing trial in the Variable delay in delivery of reward task (VDDR). Each trial begins with all lights off. The house light turns on, and after 2 seconds, the light in two random adjacent ports turns on and remains on for a maximum of 30 seconds. If a nose-poke is detected in any of the ports illuminated, the house light and the ports light turn off. After a variable delay of 0, 10, 20, 40 or 60 seconds, a light in the food cup turns on as a food pellet reward is delivered. After 5 seconds, or a nose-poke in the food cup, the light in the food cup turns off. All lights in the conditioning chamber remain off for 3 seconds before a new trial begins. Delays were presented in a random order each testing sessions, during 4 days of testing.

The following parameters were assessed during this study per each delay presented: accuracy, which reflects the nose-pokes performed in the ports that were illuminated [(correct responses/correct+incorrect responses)*100]; percentage of errors, which correspond to the nose-pokes performed in ports that were not illuminated when others were [(incorrect responses/incorrect+correct responses)*100]; omissions, reflecting trials in which animals did not perform any nose-poke when the ports were illuminated [(omitted/trials presented)*100], and latency to perform a correct nose-poke. Additionally, repetitive responses were recorded reflecting recurrent nose-poke responses made in the ports during or before the delivery of the reward [(repetitive/ repetitive+correct+errors) *100].

These daily recordings were averaged for each and the results compared between groups by two-way repeated measures ANOVA (independent variables: group and delay). These analyses were followed by post-hoc tests (Tukey).

Data were also analyzed during the training session occurring between testing sessions. The following parameter were analyzed to determine any possible interaction between tests days and re-training: accuracy ([correct responses/correct+incorrect]*100), percentage of errors ([incorrect/incorrect+correct responses]*100), omitted trials ([omitted/trials presented]*100) and latency to perform a correct nose-poke. These daily recordings were compared with a three-way repeated measures ANOVA (independent variables: group, day, and delay).

RESULTS

During training, animals in both groups nose-poked at the same rate throughout the sessions, showing a similar rate of nose-poking in the last five days of training (subjugated: 34.7 ± 3.03, control: 27.44 ± 1.90, mean ± SD). Accuracy was similar between groups (subjugated: 85.2 ± 1.3%, control: 85.6 ± 2.1%), as well as percentage of errors (subjugated: 14.8 ± 1.3%, control: 14.4 ± 2.1%) and omissions (subjugated: 54.7 ± 2.9%, control: 58.4 ± 1.8%). The latency to nose-poke after the light in the ports turned on, was also similar between groups (subjugated: 12.66 ± 0.68 seconds, control: 13.13 ± 0.41 seconds). Finally, the latency to nose-poke in the food cup within 5 seconds after a correct response was also similar between groups (subjugated: 3.10 ± 0.77 seconds, control: 3.37 ± 0.44 seconds). These rapid nose-pokes confirm the learning of the relation between nose-poke and food.

During testing, as the delay to obtain the reward got longer accuracy decreased similarly between groups, while the percentage of errors increased, in particular when the delay got longer than 10 seconds in both cases. The omissions rate was mostly similar between groups across all delays, though more omission were observed at the 10 seconds delay. Finally, the latency to respond was similar between both groups regardless of the delay presented. Accuracy rates were similar between groups [$F(1,70)= 0.211$, $p>0.05$], but decreased significantly with increasing delays [$F(4,70)= 16.474$, $p<0.001$], with lower rates at 20, 40 and 60 ($p<0.001$) seconds delay compared to 0 or 10 seconds. There was no significant interaction between group and delay for this measure [$F(4,70)= 2.454$, $p>0.05$] (Figure 18A). Alternatively, error rates were similar between groups [$F(1,70)= 0.211$, $p>0.05$], but increased significantly with longer delays [$F(1,70)= 16.474$, $p<0.001$]. Specifically, error rates were higher with 20, 40, and 60 ($p<0.001$) seconds delays than with 0 or 10 seconds. There was no significant interaction between group and delay [$F(4,70= 2.454$, $p=0.054$] (Figure 18B). Omission rates did not differ between groups [$F(1,70)= 0.827$, $p>0.05$], but varied depending on delays presented [$F(4,70)= 7.087$, $p<0.001$]. Omissions were lower with 0, 20, and 60 ($p<0.001$) seconds delay as compared to 10 seconds. There was no significant interaction between group and delay [$F(4,70)= 1.691$, $p>0.05$] (Figure 18C).

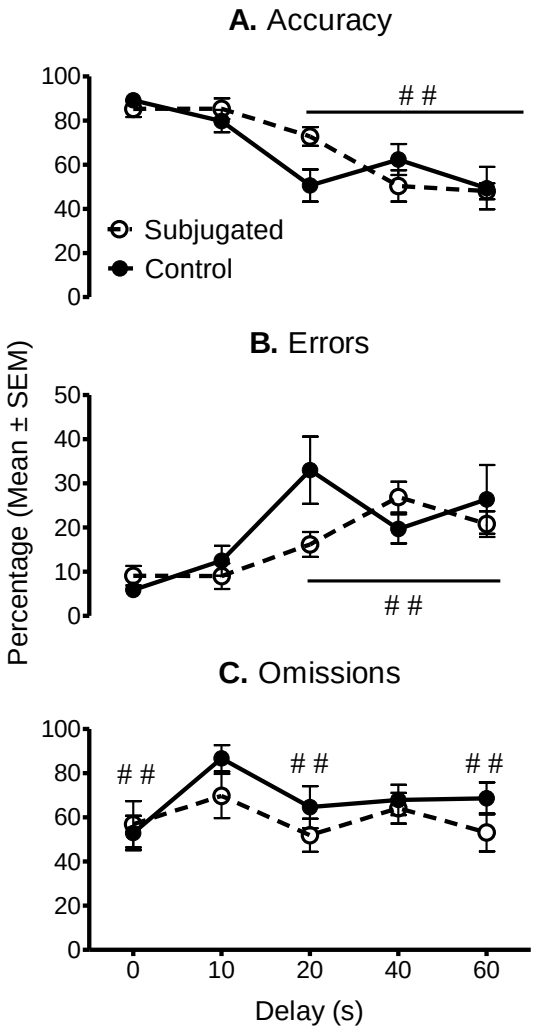


Figure 18. Results during testing in Variable delay in delivery of reward task (VDDR). A: Accuracy in VDDR. Comparison of average percentage of nose-pokes in any of two adjacent illuminated ports (ports were illuminated for 20 seconds) (##) < 0.01 with 0 and 10 second delay. B: Percentage of errors in VDRR. Comparison of average percentage of nose-pokes in ports that were not illuminated when others were. (##) < 0.01 with 0 and10 seconds delay. C: Percentage of omissions in VDRR task. Comparison of average percentage of trials in which nose-pokes were not detected in the ports during the 20 seconds respond period. (##) < 0.01 with 10 seconds delay.

Repetitive responses in this experiment correspond to repeated nose-pokes performed in any port following a successful response while waiting for the delivery of the reward. The rate of repetitive responses increased gradually with longer delays [$F(4,70)= 18.690$, $p<0.001$], the increase being significant once the delays reached 20 seconds ($p<0.001$). However, there was no significant difference between groups [$F(1,70)= 0.142$, $p>0.05$], and no significant interaction between variables [$F(4,70)= 1.209$, $p>0.05$] (Figure 19A). Finally, the latency to respond was not different between groups [$F(1,70)= 2.031$, $p>0.05$], delays [$F(4,70)= 1.213$, $p>0.005$], and did not show a significant interaction between variables [$F(4,70)= 1.118$, $p>0.005$] (Figure 19B).

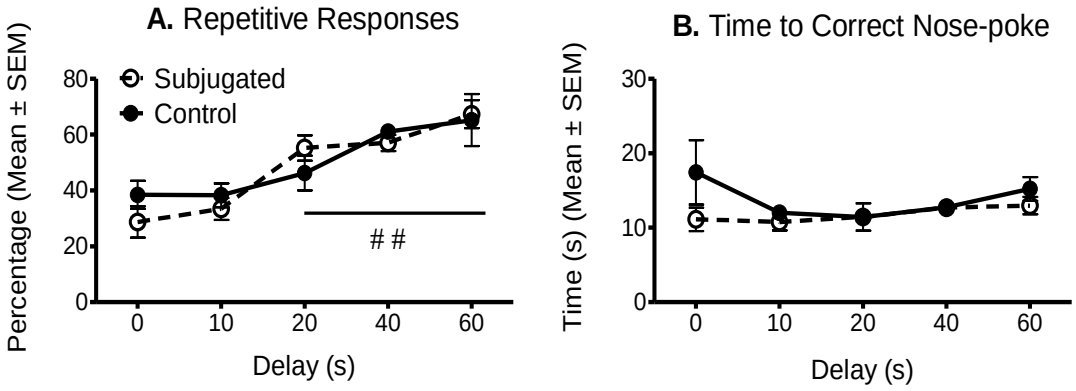


Figure 19. Results during testing in Variable delay in delivery of reward task (VDDR). A: Repetitive responses. Comparison of average percentage of nose-pokes made in the ports during the delay in the delivery of the reward. B: Latency to correct nose-poke. Comparison of average time to made a nose-poke in any of the two adjacent, illuminated ports. (##) < 0.01 with 0 and 10 s delay.

During this experiment, test days were separated by 3 days of retraining. I analyzed data from these days to determine whether the delays presented during test days could impact retraining afterwards. Overall, there was no significant main effect or interaction

between groups ($p>0.05$), day of retraining ($p>0.05$), or delay presented during testing ($p>0.05$) for any of the measures recorded (percentage of correct nose-pokes, errors, omissions, and latency to perform a correct nose-poke response).

DISCUSSION

This study was performed as a control experiment to evaluate the capacity to wait for a reward after a conditioned response, instead of waiting between two conditioning cues to initiate a response. In this study, the rates of accuracy decreased while errors increased over lengthening delays. The rates of repetitive responses increased similarly in both groups with longer delays.

Results from this experiment showed that early stress exposure does not affect the ability to wait to obtain a reward in this context. This result seems to contradict previous data (Chapter 2, Experiment 2.3). In that study, hamsters exposed to social stress in early adolescence showed an immediate inhibition in lever pressing for rewards, combined with a lack of interest in the lever after introduction of a long-delayed reward. However, there were substantial differences in the protocols used in these two studies. In the present study, animals were tested with separate trials in the conditioning chambers used for the waiting impulsivity task, marked by discrete conditioning cues and a limited time of 30 seconds to respond by nose-poking a lit opening (port). In this task animals took on average 13 seconds to respond, and the omission rate ranged between 50 and 60% of trials. In the previous study, there was no cue signaling the trials, no limited time period to lever press, and the animals were free to lever press at will during the tests. There was no omission rate as the sessions did not have discrete trials. Perhaps these procedural differences are sufficient to explain different sensitivity to delays, as they may involve different neural circuitry. One

context uses a light cue for conditioning, and the other uses contextual cues for conditioning. In fear conditioning, contextual cues vs light cues involve different neural circuits, specifically cued fear conditioning has been related to the dopaminergic system and basolateral amygdala (Fadok, Dickerson, & Palmiter, 2009; Pezze & Feldon, 2004; Maren, 2001), while context fear conditioning has been related to Hippocampus (Phillips & LeDoux, 1992; Anagnostaras, Gale & Fanselow, 2001; Selden, Everitt, Jarrari, & Robbins, 1991; Kim & Jung, 2005; Chang & Liang, 2017). Perhaps the same is possible in these tasks as well, and stress might be having differential effects on the neural systems associated, perhaps affecting more the system associated with hippocampus, than the one related to dopamine and basolateral amygdala.

Taking together, the data show that the recorded measures were selective to the timing of the delays during the testing protocols, further enhancing the selectivity of the effects of early social stress on impulsive action (waiting impulsivity). The mirror designs of these two experiments introduced delays in two different places in the conditioning procedures, addressing patience to wait to perform an action and patience to wait for a gratification. These results show that only the patience to wait to perform an action was impacted by stress in early puberty under the testing conditions, and in this case, it was improved. These data show that different aspects of patience are differentially impacted by early experience with stress. Several studies have suggested that the inability to deal with delayed rewards is related to serotonin systems. In particular, some studies have shown that the serotonergic dorsal raphe (DR) neurons are involved in the promotion of waiting for a reward, in particular when the probability of reward delivery is high (Miyazaki, Miyazaki, Yamanaka, Tokuda, Tanaka, & Doya, 2018). The differential response of subjugated animals to delayed rewards, suggest that stress might be affecting the

serotonergic system, in particular at the DR level, that could be decreasing the patience related to the ability to wait for rewards in a context-specific way, while the systems involved with patience to respond could be enhanced after stress exposure.

It is interesting that social stress in puberty in hamsters is associated with opposite effects on the two components of impulsive action: action inhibition and waiting impulsivity. These two forms of impulsivity have been associated with separate dopamine circuits in the brain (Pattij & Vanderschuren, 2008; Miyazaki, Miyazaki, & Doya, 2012; Jentsch et al., 2014; Fonseca, et al., 2015). Action inhibition has been associated with the connections of the medial striatum and its dopaminergic input from the substantia nigra. Waiting impulsivity has been associated with the connections of the nucleus accumbens and dopaminergic projections from the ventral tegmental area (VTA). Perhaps, exposure to social stress in puberty which can impact the maturation of the dopaminergic system (Watt, et al., 2014) and reduces cytochrome oxidase activity in the VTA (Bastida, et al., 2014), impacts these two dopaminergic circuits differentially.

In summary, exposure to social stress in early puberty has opposite effects on the components of impulsive action: decreased capacity to inhibit a response (Chapter 2, Experiment 2.1) and enhanced capacity to wait before initiating an action (this study).

CHAPTER 4: STRESS AND PERSEVERANCE

Exposure to early trauma has been related to some psychopathologies, such as Bipolar disorder (Etain, Henry, Bellivier, Mathieu, & Leboyer, 2008) and Borderline personality disorder (Herman & Perry, 1989). Even though these disorders share characteristics such as aggression and impulsivity, perseverance seems to be a differential factor between them. Some studies have shown differences in perseverance between these psychopathologies, with Borderline showing higher lack of perseverance compared to Bipolar (Bøen, et al., 2015), while some others have not shown differences between these two disorders (Shafiee-Kandjani, et al., 2017). This lack of consistency suggests the existence of different subtypes of aggressive/impulsive profiles. In addition, as mentioned before, hamsters exposed to subjugation during adolescence show enhanced aggression (Wommack, et al., 2013), and impulsive action as adults (Chapter 2). However, these animals showed different responses to a delayed in the delivery of the reward, decreased response in one context and no changes in response on a different context (Experiment 2.3 and Experiment 3.2). Therefore, I decided to test perseverance in the same context in which previously stressed animals showed decreased response in the presence of a delay compared to controls (Experiment 2.3).

Testing perseverance can broaden the aggression/impulsivity profile observed in animals exposed to early social stress. For this, I am evaluating animals' response to increases in the physical effort required to obtain food pellet rewards and to changes in the probability of reward. Thus, the willingness to exert more effort and to continue responding to obtain a reward, will provide a measure of perseverance as the ability to follow through the task in spite of the increased difficulty.

EXPERIMENTAL DESIGN

Two sets of 14 animals were used for these experiments. Each set was distributed into subjugated and control groups (n=7 per group) as explained above (Experiment 2.1), for a total of 28 animals, with n=14 per group. Animals in the first set, were trained and tested in an effort task, followed by 2 weeks of rest, 4 days of retraining, and then tested in a probability task. The second set of animals followed the same timeline, but the order of the task was reverted, therefore they were tested in the probability task first, and the effort task second (Figure 20).

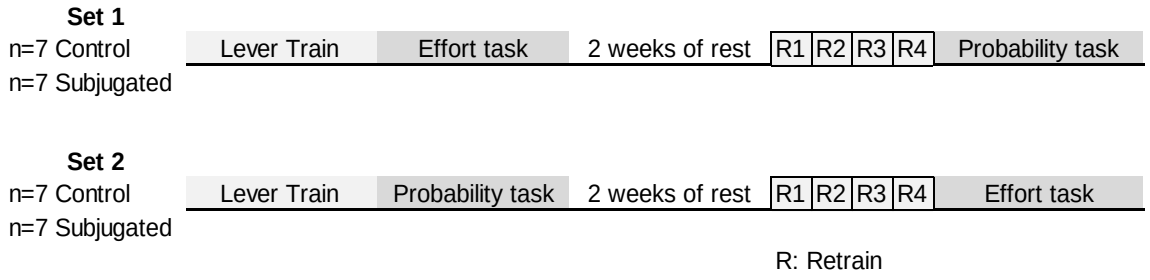


Figure 20. Timeline representation of perseverance experiments. Time line representation of the training and testing for two sets of 14 animals used to test perseverance in effort and probability tasks.

On P60, after magazine training, all animals were trained to lever press an active lever to receive food pellet rewards in the same protocol as described before (Experiment 2.3). An inactive lever was present, but lever presses in this lever did not have any consequence associated. Once proficient lever pressing was observed, animals were tested in the effort requirement task or the probability task.

Testing on the first task was followed by two weeks of rest before animals were exposed to 4 session of retraining to the same training protocol used during lever press training, before being tested for the second experiment.

During training and retraining before testing the following parameters were recorded: frequency of lever presses in the active and inactive lever, latency to nose-poke after lever pressing the active lever, and nose-poke within 10 seconds after food pellet delivery. The number of active lever presses, and time to nose-poke was use as indicators of learning the association between lever pressing and food pellet rewards.

To determine that there were no differences between groups and sets during training or retraining these measures were analyzed with three-way repeated measures ANOVA [independent variables: group, set and day of training]. These analyses were followed by t-test between variables involved in significant main effects or interactions. For the t-test comparison the alpha value was adjusted by dividing the standard p value = 0.05 over the number of t-test performed.

Results for lever training and retraining before testing

Lever press acquisition

Both groups of animals (control and subjugated) learned to lever press for food pellet rewards, but there were differences between sets in lever presses in the active lever, without differences in inactive lever presses. In the frequency of lever presses in the active lever there was a significant main effect of set [$F(1,24)= 18.721$, $p<0.05$], without any other significant main effect or interactions ($p>0.05$). The first set of animals (21.07 ± 6.691, mean ± SE) compared to the second set of animals (62.017 ± 6.691) had significantly fewer lever presses in the active lever [$t(24)= -4.327$, $p<0.01$]) (Figure 21A). During the last 4 days of training lever presses in the inactive lever was different between groups [$F(1,24)= 6.322$, $p<0,05$], without any other significant main effect or interaction ($p>0.05$). Particularly, subjugated (6.892 ± 0.969) animals had a higher frequency of lever presses in

the inactive lever compared to controls (3.446 ± 0.969) [$t(24)= 2.514$, $p<0.05$] (Figure 21B). However, it is important to mention that these are lever presses in the inactive lever, and are very low in comparison with the active lever presses. When observing the animals in the conditioning chamber, it has been observed that these presses are not followed by a nose-poke in the food cup and most of the time are triggered by the animals jumping around in the corner, or biting the metal grid below the lever, which suggest that these are not intentional lever presses.

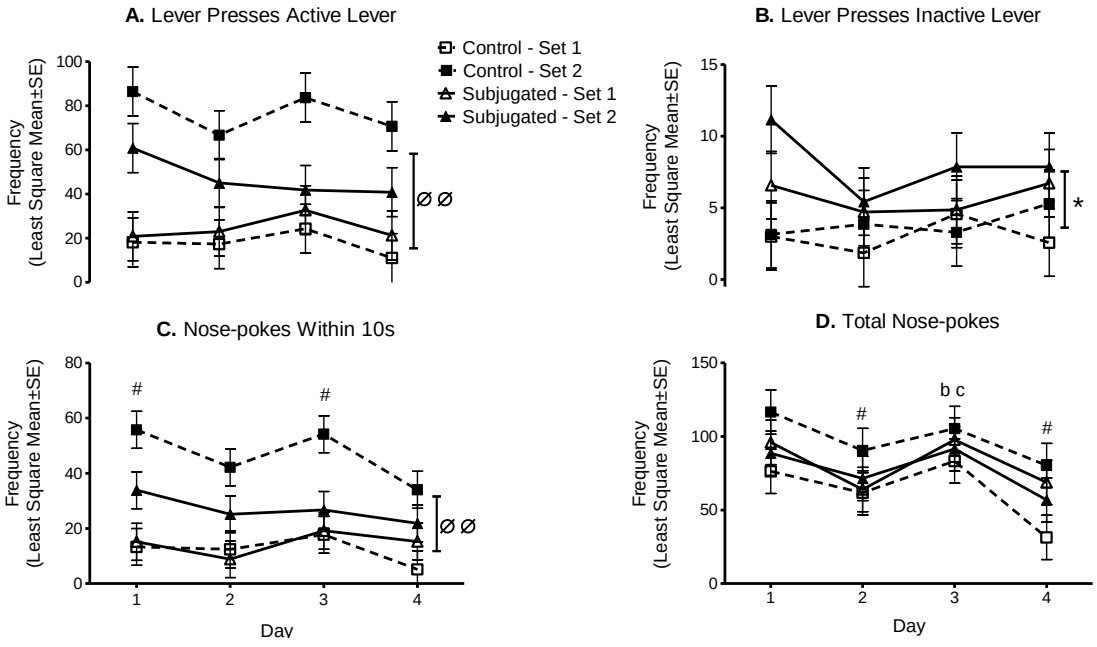


Figure 21. Results of last four days of training in lever press acquisition. Two sets of n=14 animals were divided between control=7 and subjugated=7 and trained to lever press for food pellet rewards before testing in the first task. A: Frequency of lever presses in the active lever. Comparison of average frequency of lever presses to obtain food pellet rewards. (ØØ) <0.01 between sets. B: Frequency of lever presses in the inactive lever. Comparison of average frequency of lever presses in the lever not associated with food pellet rewards. (*) <0.05 between groups. C: Nose-pokes in the food cup within 10 seconds. Comparison of average frequency of nose-pokes in the food cup within 10 seconds after pellet being delivered. (ØØ) <0.01 between sets. (#) < 0.05 compared to day 4. D: Total nose-pokes in the food cup. Comparison of total nose-pokes in the food cup across days of testing. (#) < 0.05 compared to day 1. (b) <0.05 compared to day 2. (c) < 0.05 compared to day 4.

There were no significant main effects or interactions in the time to nose-poke in the food cup to retrieve the reward ($p>0.05$). However, there was a significant effect of set [$F(1,24)= 18.014$, $p<0.05$] and day [$F(3,72)=5.783$, $p<0.05$] in the frequency of nose-pokes in the food cup within 10 seconds of pellet delivery. Particularly, the first set of animals (13.375 ± 3.8853) compared to the second set of animals (36.696 ± 3.885) had significantly

fewer nose-pokes in the food cup within 10s [$t(24)= -4.244$, $p<0.01$]. In the analysis of the main effect of day, it was observed that the nose-pokes within 10 seconds in day 1 (46.571 ± 5.545) and day 3 (45.642 ± 5.545) were significantly higher than in day 4 (35.964 ± 5.545) ([$t(72)= 2.247$, $p<0.05$] and [$t(72)= 2.050$, $p<0.05$] respectively) (Figure 21C). Finally, there was a significant main effect of day in the total frequency of nose-pokes in the food cup [$F(3,72)= 7.774$, $p<0.05$], without any other significant main effect or interactions ($p>0.05$). In particular, the frequency of total nose-pokes in day 1 (94.357 ± 7.525) was significantly higher compared to day 2 (71.928± 7.525) and day 4 (59.357 ± 7.525) ([$t(72)= 2.542$, $p<0.05$] and [$t(72)= 3.967$, $p<0.05$] respectively). Additionally, day 2 compared to day 3 (94.5 ± 7.525) was significantly lower [$t(72)= -2.559$, $p<0.05$], and day 3 compared with day 4 was significantly higher [$t(72)= 3.984$, $p<0.05$] (Figure 21D).

Retraining before testing

I also analyzed the parameters recorded during the 4 days of retraining before the second task presented. In this case both groups (control and subjugated) recover the lever pressing behavior in the active lever, without significant differences in the inactive lever pressing. The analysis of frequency of lever presses in the active lever showed a significant main effect of set [$F(1,24)= 9.409$, $p<0.05$], without any other significant main effect or interaction ($p>0.05$). Frequency of lever presses in the active lever of the first set of animals (35.6785 ± 9.599) compared to the second set (77.321 ± 9.599) was significantly lower [$t(24)= -4.327$, $p<0.01$] (Figure 22A). There were no significant main effects ($p>0.05$) or interactions ($p>0.05$) in the frequency of lever presses in the inactive lever (Figure 22B), or time to nose-poke in the food cup to retrieve the food pellet rewards.

On the parameter of nose-pokes in the food cup within 10 seconds of pellet delivery there was a significant effect of set [$F(1,24)= 12.8843$, $p<0.05$] and a significant interaction between set and day [$F(3,72)=3.5827$, $p<0.05$]. Frequency of nose-pokes within 10s was significantly lower in the first set of animals (17.5714 ± 5.2705) compared to set 2 (44.14 ± 5.2705) in day 1 [$t(58.14)= -3.656$, $p<0.01$], day 2 [$t(58.14)= -3.919$, $p<0.01$] (set 1: 11.1428 ± 5.270; set 2: 40.3517 ± 5.270) and day 3 [$t(58.14)= -2.808$, $p<0.01$] (set 1: 21.071 ± 5.270; set 2: 42 ± 5.270) (Figure 22C). Finally, there was a significant main effect of set in the total frequency of nose-pokes in the food cup [$F(1,24)= 12.1352$, $p<0.05$] without additional main effects of significant interaction ($p<0.05$). The first set of animals (64.1964 ± 8.9675) had significantly fewer nose-pokes in the food cup than the second set (108.375 ± 8.9675) [$t(24)= -3.484$, $p<0.01$] (Figure 22D).

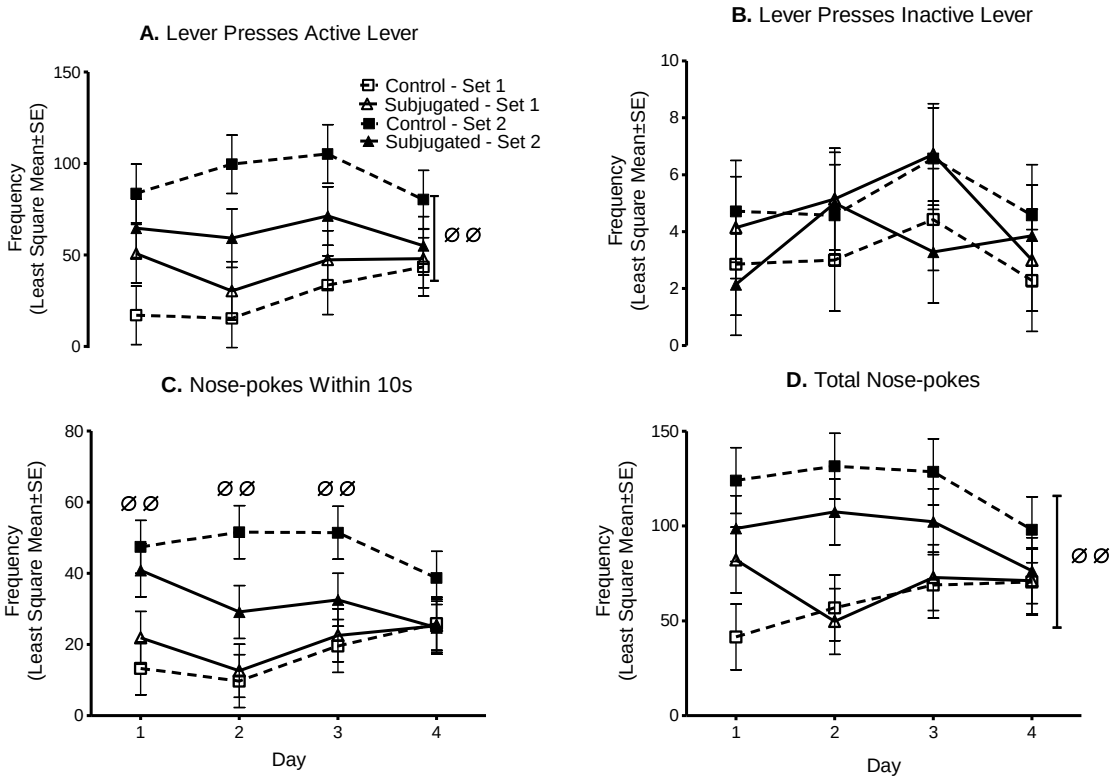


Figure 22. Results of four days of retraining before second test of perseverance. Two sets of n=14 animals divided between control=7 and subjugated=7 were exposed to the training protocol before testing on the second task. A: Frequency of lever presses in the active lever. Comparison of average frequency of lever presses in the lever associated with food pellet rewards. B: Frequency of lever presses in the inactive lever. Comparison of average frequency of lever presses in the lever not associated with food pellet rewards. C: Nose-pokes in the food cup within 10 seconds. Comparison of total nose-pokes in food cup within 10 seconds of food delivery. D: Total nose-pokes in the food cup. Comparison of total nose-pokes in the food-cup. (ØØ) <0.01 between sets.

Experiment 4.1: Changes in effort requirement

EXPERIMENTAL DESIGN

Two sets of 14 animals, were distributed into subjugated and control groups (n=7 per group) as explained before (Experiment 2.1), for a total of 28 animals, with n=14 per group. After lever train or retrain before testing, animals were tested each day to a unique requirement of 1, 2, 4, 8 or 16 lever presses to obtain food pellets. The requirement for each day of testing was randomly selected, controlling the possible effect of testing in an increasing or decreasing order of the requirement. Due to the results obtained in Experiments 2.2 and 2.3 in which the absence of an immediate reward significantly decreased lever pressing, and in the present experiment it was possible that the animals experience and absence of the reward if the requirement was not met, animals were exposed to 3 sessions of training during 3 consecutive days after each testing session. With this, if during testing sessions the animals did not meet the requirement and therefore did not receive any food pellet rewards, the lever pressing behavior was recovered through three days of retraining before the next day of testing. The procedure of these training session was the same as the original protocol used during training phase. Pilot studies have shown that three days of retraining between testing session is enough to recover the response.

During this study the following parameter were recorded: frequency of lever presses in the active and inactive lever, times that the criteria was met, frequency of nose-pokes within 10 seconds after food pellet delivery, total nose-pokes in the food-cup, and total lever presses in the active lever per requirement presented. The number of active lever presses, and the frequency of nose-pokes within 10 seconds were used as indicators of learning of the association between lever pressing and food pellet rewards. These parameters, and latency to nose-poke in the food cup to retrieve food pellets, were also

recorded during re-training sessions to confirm its efficacy and ascertain any possibility of interactions between testing and re-training, which could in turn impact the next testing session.

The measures were analyzed with three-way repeated measures ANOVA [independent variables: group, set, and requirement tested]. These analyses were followed post-hoc t-test between variables involved in significant main effects or interactions. The data for retraining sessions was analyzed using three-way repeated measures ANOVA [independent variables: group, day of retraining, requirement tested] and followed by post-hoc test t-test between variables involved in the interactions or main effects. For the t-test comparison the alpha value was adjusted by dividing the standard p value = 0.05 over the number of t-test performed.

RESULTS

Testing

Because the training results were discussed earlier, in this section I am reporting the results for testing and retraining in between testing session. One animal in the control group was not tested for the 4 lever presses requirement, and due to his missing data for that requirement he was excluded from the data analyses in the test session. Thus, the analyses for testing included n=13 control and n=14 subjugated.

During testing, both groups decrease the frequency of times in which the required number of lever presses was achieved, the frequency of lever presses in the inactive lever, nose-pokes in the food cup within 10 seconds of the pellet being delivered, without changes in the latency to retrieve the reward. The analysis of the frequency of times animals lever pressed the required amount of times showed a significant main effect of set [$F(1,23)=$

6.2161, $p<0.05$], effort [$F(4,92)= 36.3010$, $p<0.05$], a significant interaction of set and effort [$F(4,92)= 3.7434$, $p<0.05$], group and effort [$F(4,92)= 2.5052$, $p<0.05$] and set, by group by effort [$F(4,92)= 4.0395$, $p<0.05$]. The frequency of trials with requirement met in the first set of animals was significantly lower compared to the second set of animals in particular in the control group ($p<0.01$) at the level of 1 lever press requirement ($p<0.01$) (Figure 23A). Additionally, in the main effect of effort, the frequency of criteria met in the 1 lever press requirement was significantly higher than when it was 2, 4, 8 or 16 lever presses ($p<0.01$), and at 2 lever presses compared to 8 and 16 lever presses ($p<0.01$) (Figure 16A). In the frequency of inactive lever presses during testing, there was a significant main effect of effort [$F(4,92)= 4.8190$, $p<0.05$], without additional main effects or interactions ($p>0.05$). The number of lever presses in the inactive lever was significantly higher when the requirement was 16 lever press than when it was 1, 2, or 4 lever presses ($p<0.01$). Additionally, the lever presses in the inactive lever when the requirement was 4 lever presses was significantly lower than when it was 8 lever presses ($p<0.05$) (Figure 23B). There were no significant main effects ($p>0.05$) or interactions ($p>0.05$) in the latency to retrieve the food pellet rewards.

The frequency of nose-pokes within 10 seconds to retrieve the reward significantly decreased as the effort got higher. The analysis showed a significant main effects of set [$F(1,23)= 5.5275$, $p<0.05$] and effort [$F(4,92)= 24.3589$, $p<0.05$]. The second set of animals had significantly more nose-pokes within 10s compare to the first set of animals ($p<0.05$). Additionally, the frequency of these nose-pokes when the requirement was 1 lever press, was significantly higher than when the requirement was 2, 4, 8, or 16 lever presses ($p<0.01$). Moreover, the frequency of these nose-pokes was higher in the 2 lever presses requirement compared to 8 and 16 ($p<0.01$), and in 4 lever presses compared to 8

($p<0.05$) and 16 ($p<0.01$) (Figure 23C). The total frequency of nose-pokes showed a significant main effect of effort [$F(4,92)= 4.4090$, $p<0.01$], without other significant main effects or interactions ($p>0.05$). Total nose-pokes were higher at the 1 lever presses requirement compared to 2 ($p<0.01$), 4 ($p<0.05$), 8 or 16 lever presses ($p<0.01$).

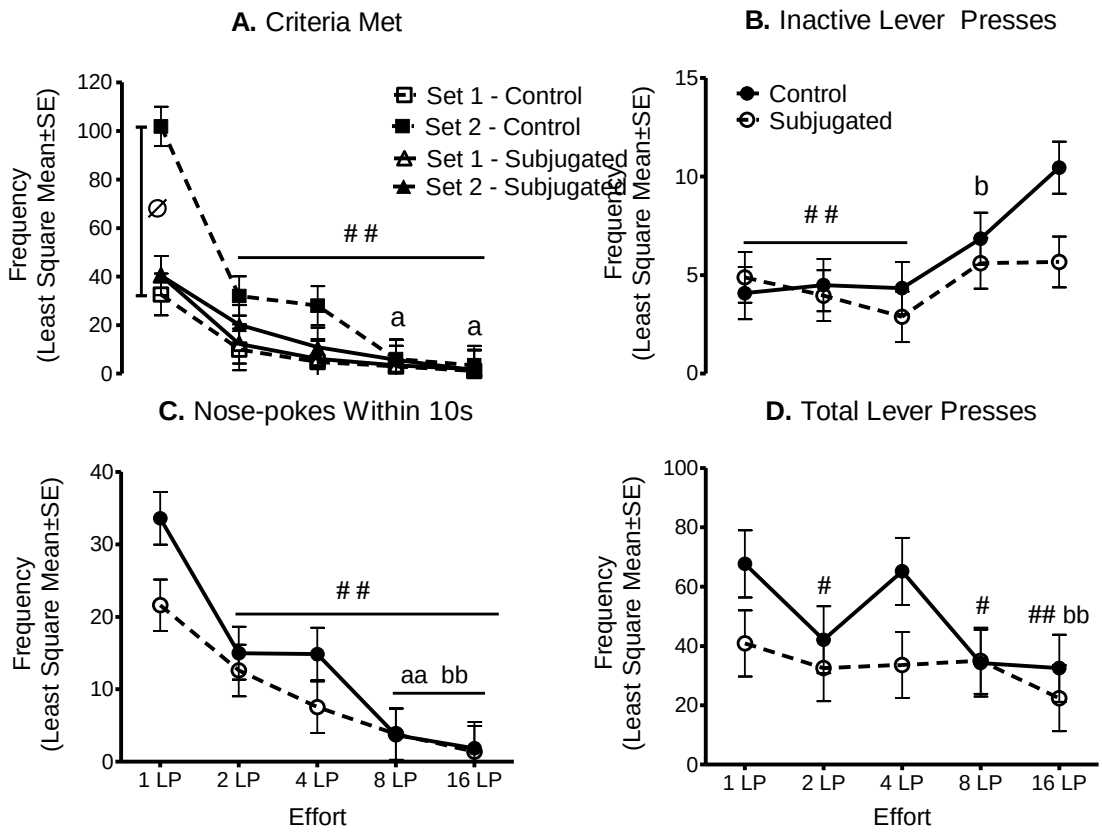


Figure 23. Results during testing in Effort task. If in the three-way repeated measures ANOVA there was not a significant interaction between set, group and effort, the data for the two sets is combined on each group for representative purposes. A: Criteria met Comparison of average frequency of trials in which the required lever presses were made, across requirements tested. (Ø) < 0.01 between Set1 and Set 2 in Control group at 1LP. (##) < 0.001 compared with 1LP. (a) < 0.05 compared with 2LP. B: Lever presses in the inactive lever. Comparison of average lever presses in the lever not associated with food pellet rewards across lever pressing requirements tested. (##) < 0.001 compared with 1LP. (b) <0.05 compared with 4LP. C: Nose-pokes within 10 seconds. Comparison of average frequency of nose-pokes in the food cup within 10 seconds of pellets delivery. (##) < 0.001 compared with 1LP. (aa) <0.01 compared with 2LP. (bb) <0.01 compared with 4LP. D: Total lever presses. Comparison of total lever presses in the active lever across requirements tested. (##) < 0.001 compared with 1LP. (#) < 0.05 compared with 1LP. (bb) <0.01 compared with 4LP.

Finally, the number of total lever presses per requirement presented showed a significant main effect of set [$F(1,23)= 6.4748$, $p<0.05$] and effort [$F(4,92)= 3.8428$, $p<0.01$]. Further analyses indicated that this parameter was higher in the second set of animals compared to the first ($p<0.05$). Additionally, the total lever presses per requirement was higher when the requirement was 1 lever press compared to 2, 8 ($p<0.05$), and 16 lever presses ($p<0.01$), and when it was 4 lever presses compared to 16 lever presses ($p<0.01$) (Figure 23D).

Retraining between testing sessions

During this experiment, test days were separated by 3 days of retraining. I analyzed data from these days to determine whether the effort requirements presented during test days could impact retraining afterwards. Because during lever press acquisition there were no significant interactions between set and group, I decided to combine both sets of animals on each group (control and subjugated) to analyze the retraining between testing sessions. Overall, there were significant main effects or interaction between groups ($p>0.05$), day of retraining ($p>0.05$), or requirement presented during testing ($p>0.05$) for the lever presses in the active lever or the latency to nose-poke in the food cup to retrieve food pellet rewards. The analysis of frequency of lever presses in the inactive lever during retraining did not show significant main effects of group, requirement tested, day of retraining ($p>0.05$), or first order interactions ($p>0.05$). However, there was a significant interaction between group, requirement tested, and day of retraining [$F(8,208)= 2.710$, $p<0.05$]. The frequency of lever presses in the inactive lever in the first day of retraining was lower than in the second day, in the control group at the level of 1 lever press requirement [$t(93)= -352$, $p<0.01$], and the first day of retraining compared to the third day of retraining in subjugated

animals at 2 lever presses requirement [$t(93)= -2.640$, $p<0.01$]. It is important to mention that this interaction is in the frequency of lever presses in the inactive lever, which is not associated with any consequence. Observations of the animals in the conditioning chambers has shown that the majority of this lever presses are not followed by a nose-poke in the food cup, and most of the time correspond to the animals jumping near this lever, or biting the grid under the lever and accidently pressing the lever. The more relevant measure is frequency of lever presses in the active lever, which is the one associated with the delivery of food pellets, and in this case, there are no significant differences or interactions in this measure.

Experiment 4.2: Changes in probability of the reward.

EXPERIMENTAL DESIGN

Two sets of 14 animals, were distributed into subjugated and control groups (n=7 per group) as explained above (Experiment 2.1), for a total of 28 animals, with n=14 per group. After lever press acquisition or retrain before testing (Figure 20), animals were tested for 4 days to changes in the probability of obtaining food pellet rewards. Animals were tested each day to a unique probability of 12, 25, 50 or 100 % probability to obtain food pellets. The probability for each day of testing was randomly selected, controlling the possible effect of testing in an increasing or decreasing order. Due to the results obtained in Experiments 2.2 and 2.3 in which the absence of an immediate reward significantly decreased lever pressing, and in the present experiment it was possible that the animals experience and absence of the reward for each lever press, animals were exposed to 3 sessions of training during 3 consecutive days after each testing session. With this, if during testing sessions the animals are not rewarded for every lever press and for this reason the response decreases, the lever pressing behavior was recovered through three days of retraining before the next day of testing. The procedure of these training session was be the same as the original protocol used during training phase.

During this study the following parameter were recorded: frequency of lever presses rewarded, frequency of lever presses in the active and inactive lever, total nose-pokes in the food-cup, and frequency of nose-pokes within 10 seconds after receiving a food pellet reward. The number of active lever presses, and time to nose-poke were used as indicators of learning of the association between lever pressing and food pellet rewards. I also recorded these parameters, and latency to nose-poke in the food-cup to retrieve food pellet rewards, during re-training sessions to confirm its efficacy and ascertain any

possibility of interactions between testing and re-training, which could in turn impact the next testing session.

The measures were analyzed with three-way repeated measures ANOVA [independent variables: group, set and probability]. These analyses were followed post-hoc t-test between the variables involved in the significant main effects or interactions. The measures for retraining sessions were analyzed using three-way repeated measures ANOVA [independent variables: group, day of retraining, probability tested], and followed by post-hoc analysis t-test between the variables involved in the significant main effects or interactions. For the t-test comparison the alpha value was adjusted by dividing the standard p value = 0.05 over the number of t-test performed.

RESULTS

Testing

Because the training results were discussed earlier, in this section I am reporting the results of testing and retraining in between testing session.

During testing both groups increased the frequency of lever presses rewarded with as the probability of reinforcement got higher, without changes in the total lever presses in the active or inactive lever. The analysis of the frequency of lever presses rewarded indicated a significant main effect of probability [$F(3,72)= 34.6011$, $p<0.01$], without any other significant main effect ($p>0.05$) or interaction ($p>0.05$). Lever presses rewarded at 100% probability were significantly higher than the 12, 25 and 50% probabilities ($p<0.01$). Additionally, the lever presses rewarded at the 12% probability were lower than at 50% ($p<0.01$), and the ones at 25 than those at 50% ($p<0.01$) (Figure 24A). In the frequency of total lever presses in the active lever the analysis showed a significant main effect of set

[$F(1,24)= 7.5963$, $p<0.05$] and a significant interaction between set and group [$F(1,24)= 5.337$, $p<0.05$], without any additional significant main effects ($p>0.05$) or interactions ($p>0.05$). There was a significant difference between sets in the control group ($p<0.01$), in particular the overall lever presses in the active lever was lower in the first set of animals compared to the second set [$t(24)= -3.583$, $p<0.01$) (Figure 24B). In the frequency of lever presses in the inactive lever the analysis indicated a significant main effect of set [$F(1,24)= 11.6336$, $p<0.01$], without additional significant main effects ($p>0.05$) of interaction ($p>0.05$). In particular, the frequency of lever presses in the inactive lever in the first set of animals was lower compared to the second set [$t(24)= -3.411$, $p<0.01$] (Figure 24C).

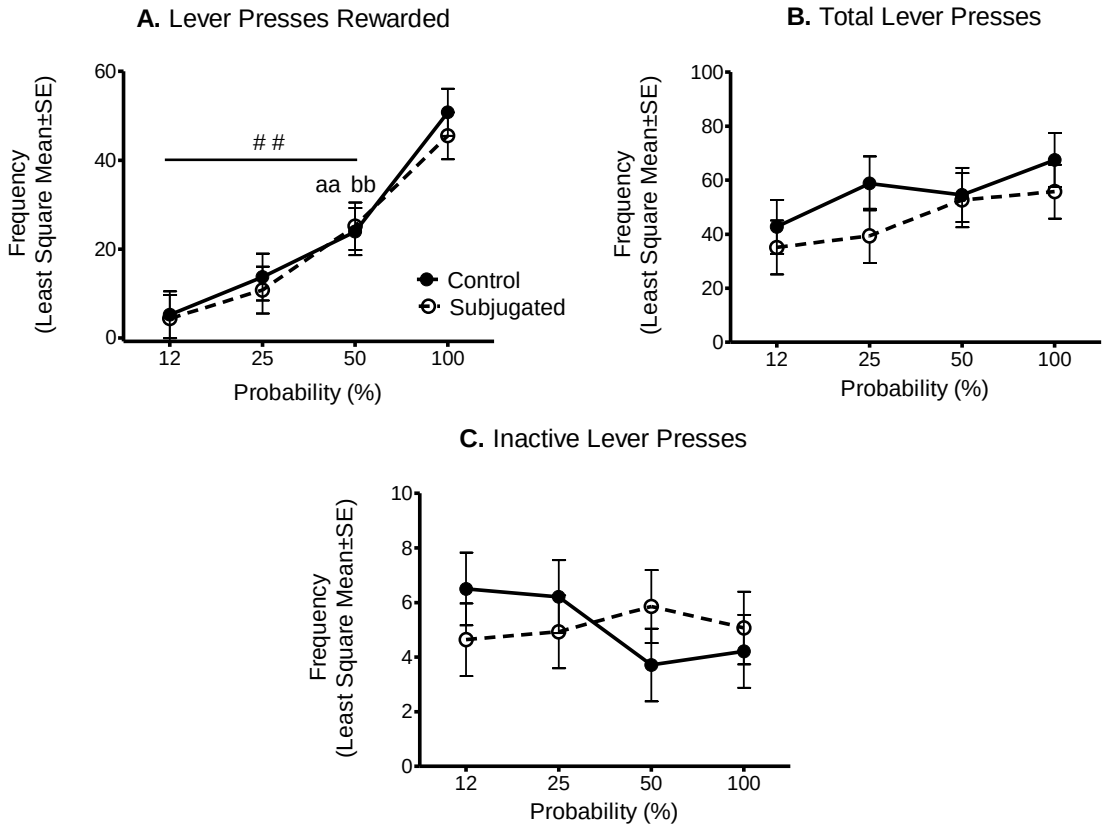


Figure 24. Results during testing in Probability task. If in the three-way repeated measures ANOVA there was not a significant interaction between set, group and probability, the data for the two sets is combined on each group for representative purposes. A: Total lever presses rewarded. Comparison of total lever presses that were rewarded across probabilities of reinforcement. (##) < 0.01 compared with 100%. (aa) <0.01 compared with 12%. (bb) <0.01 compared with 25% B: Lever presses in the active lever. Comparison of total lever presses in the lever associated with food pellet rewards across probabilities of reinforcement. C: Total lever presses in the inactive lever. Comparison of total lever presses in the lever not associated with food pellet rewards across probabilities of reinforcement

The analysis of overall nose-pokes in the food cup indicated a significant main effect of probability [$F(3,72)= 3.0704$, $p<0.05$], without additional significant main effects ($p>0.05$) or interactions ($p>0.5$). Nose-pokes at the 100% probability were higher than at

12% ($p<0.01$), and 50% probability ($p<0.05$) (Figure 25A). The frequency of nose-pokes within 10s in the food-cup to retrieve the food pellet indicated a significant main effect of probability [$F(3,72)= 22.4069$, $p<0.01$] without any additional significant main effects ($p>0.05$) or interactions ($p>0.05$). The nose-pokes in the food cup within 10s at the 12% probability was significantly lower than at 25 ($p<0.05$), 50 or 100% probability ($p<0.01$). Additionally, this parameter was lower at 25% compared to 50% ($p<0.05$), and 100% probability ($p<0.01$), and at 50% compared with 100% probability ($p<0.01$) (Figure 25B).

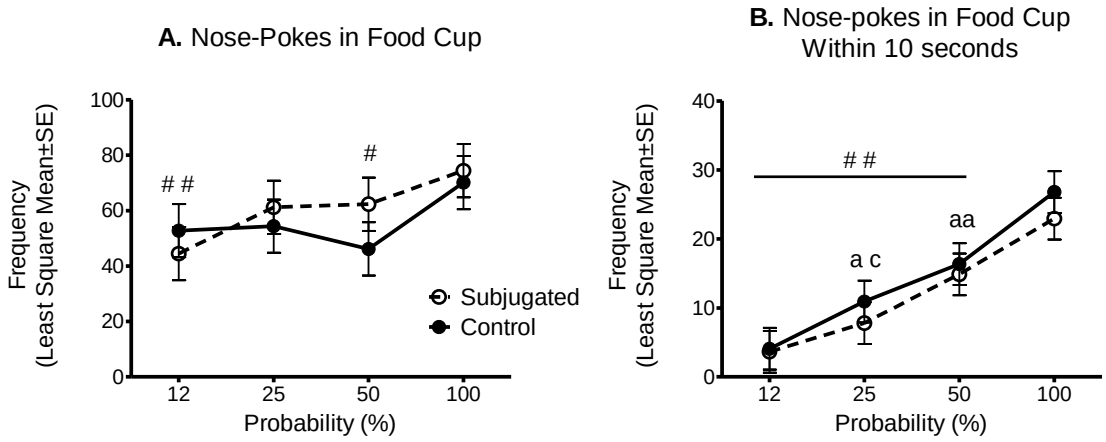


Figure 25. Results during testing in Probability task. If in the three-way repeated measures ANOVA there was no significant interaction between set, group and probability, the data for the two sets is combined on each group for representative purposes. A: Nose-pokes in the food cup. Comparison of total nose-pokes in the food cup across probabilities of reinforcement. (##) < 0.01 compared with 100%. (#) < 0.05 compared with 100%. B: Nose-pokes in the food cup within 10 seconds. Comparison of total nose-pokes in the food cup within 10 seconds of pellet delivery across probabilities of reinforcement (##) < 0.01 compared with 100%. (aa) <0.01 compared with 12%. (a) <0.05 compared with 12%. (c) < 0.05 compared with 50%.

Retraining between testing sessions

During this experiment, test days were separated by 3 days of retraining. I analyzed data from these days to determine whether the probabilities presented during test days could impact retraining afterwards. Because during task acquisition, and testing there were no significant interactions between set and group, I decided to combine both sets of animals on each group (control and subjugated) to analyze the retraining between testing sessions. Overall, there was no significant main effect or interaction between groups ($p>0.05$), day of retraining ($p>0.05$), or probability of reinforcement presented during testing ($p>0.05$) for any of the measures recorded.

DISCUSSION

The main goal of these experiments was to evaluate whether exposure to social stress in early adolescence causes long-term deficits in perseverance (e.g. the ability to follow through the task in spite of the increased difficulty). This possibility was tested with two tasks that evaluated animal's responses to changes in effort requirements and changes in the probabilities to receive a reward. Animals in control and subjugated groups show similar perseverance in lever pressing response across tasks.

To test perseverance with changes in the effort required to obtain a reward I used a modified version of the Between-session progressive ratio task (Czachowski & Samson, 1999) adapted to hamsters. In the present experiment, animals encountered variable requirements of lever pressing to obtain a single food pellet reward. The most relevant measure of perseverance was the total number of lever presses per requirement presented. Both groups equally decreased the overall lever pressing per requirement as the effort to obtain the reward got higher. Consequently, exposure to stress during early adolescence does not impair perseverance when more psychical effort is required to obtain a reward.

Traditionally, Progressive ratio tasks (PrR) are used to determine the "break point", or limit, to the amount of work that a subject is willing to perform to obtain a reinforcer (Czachowski & Samson, 1999). The amount of effort willing to perform is inferred from the break point values, which are typically measured in a single session by increasing the number of responses required for successive reinforcer presentations (Hodos, 1961). However, because PrR tasks have been used mostly with stimulant drugs, a concern has raised of possible confound effects of drug intake on subsequent responses. Therefore, an alternative procedure of Between-Sessions Progressive ratio task has been suggested. In the Between Session PrR task a progressive ratio procedure is used, but in this case increase response requirements are presented across single daily sessions (Czachowski & Samson, 1999). Since the response rate of hamsters observed in previous studies is around 50%, in order to collect enough data for each animal, I decided that it was more appropriate to adapt the Between Sessions PrR task and individually present random lever presses requirements on each testing session. Additionally, I followed each testing session with three re-training sessions to recover the lever pressing behavior to baseline levels.

Not many studies have evaluated the effect of stress on perseverance using the PrR or Between Sessions PrR tasks and the evidence is not consistent. One study showed that adult and adolescent male Long-Evans rats exposed to social defeat, did not differ from control animals in a nicotine self-administration PrR task (Zou, Funk, Shram & Lê, 2014). The same effect has been observed in adult male Long-Evans rats exposed to social defeat and tested in cocaine or heroin self-administration PrR tasks (Covington & Miczek, 2001; Yap, Chartoff, Holly, Potter, Carlezon, & Miczek, 2014; Cruz, Quadros, Hogenelst, Planeta & Miczek, 2011).

Other studies have shown different effects, suggesting increased perseverance after social defeat exposure. In a study with male adult Long-Evans rats, the breaking point of cocaine intake was significantly increased by social defeat stress in a PrR task (Covington & Tropea, 2008; Covington & Miczek, 2005). In a separate study, adolescent male Long-Evans rats exposed to social defeat showed an increased breaking point in the PrR task compared to controls when tested as adults in a cocaine self-administration task (Burke & Miczek, 2015). It is important to highlight that the studies presented have been performed with Long-Evans rats, which as mention earlier (chapter 1), present a different pattern of HPA axis maturation compared to humans and hamsters. In addition, these studies use reinforcers with higher reward values (nicotine, cocaine, and heroin) compared to the ones used in my experiment (banana pellets), especially when animals are not food deprived like in the case of my tasks. These two major differences, animal models, and reinforcer used, in addition to the methodological differences between the PrR tasks used in these studies and the adaptation of Between Sessions PrR that I used, difficult the comparison of these results with the ones obtained in the present studies.

As previously mentioned, some mental disorders are characterized by aggression and impulsivity, and are related to early stress or trauma exposure. Two of these disorders are depression and bipolar disorder. These disorders are characterized by anhedonia, which can refer to the experience of reward and motivated behavior to obtain a reward (Der-Avakian, & Markou, 2011). To my knowledge, there is only one study that have used a quantitative measure of perseverance in clinical population. This study used the PrR task to evaluate the motivated behavior to obtained a reward in unipolar, bipolar and control participants. The results showed that depressed participants (unipolar and bipolar)

exhibited lower break points compared to controls, suggesting decreased perseverance compared to control/healthy participants (Hershenberg, et al., 2016).

Perseverance has been studied in clinical populations using inventories like the UPPS Impulsivity Scale, which has four subscales: Urgency, lack of Premeditation, lack of Perseverance, and Sensation Seeking. Using this scale, it has been observed that individuals with borderline personality disorder (BPD), pathological gamblers (PG), and individuals with alcohol abuse (AA) problems, had the highest scores in lack of perseverance (but did not differ from each other), and only BPD had higher lack of perseverance compared to control/healthy participants (Whiteside, Lynam, Miller & Reynolds, 2005). In the same way, a separate study also found that BPD participants and bipolar II patients also exhibited significantly elevated lack of perseverance scores compared to controls, but BPD had higher scores compared to bipolar (Bøen, et al., 2015), supporting the idea of lower perseverance in these clinical populations.

It is clear that the evidence in relation to perseverance is not consistent. However, as shown by Jacob, Gutz, Bader, Lieb, Tüscher and Stahl (2010), self-reports can differ from behavioral data. Therefore, it would be necessary to have more studies that measure quantitively perseverance in clinical populations, in particular in those disorder related to stress.

To test perseverance through changes in the probability to obtain a reward I used a modified version of random-ratio schedule of reinforcement, in which the probability of reinforcement is the same for all responses (Sidley & Schoenfeld, 1964). I considered this schedule of reinforcement more appropriate to test perseverance than the variable-ratio schedules commonly used to model gambling-like behaviors, in which although it is possible to specify the mean and distribution of the number of responses required until a

reinforcement occurs, the probability of reinforcement is not necessarily the same for every response (Sidley & Schoenfeld, 1964; Madden, Ewan, & Lagorio, 2007). Since the response rate of hamsters in previous experiments is around 50%, in order to collect enough data for each animal I decided that it was appropriate to present a unique probability that determined the probability of reinforcement each session. Additionally, each testing session was followed by three consecutive retraining days. Unfortunately, to my knowledge there are no studies that have used random-ratio schedule of reinforcement to test animals after stress exposure. Some studies using probability have tested animals with the probability-discounting task. In particular, Wistar adult rats exposed to acute or chronic social defeat did not differ from control animals in this probabilistic task (Boutros, Der-Avakian, Semenova, Lee, & Markou, 2016). In a separate study, testosterone-treated rats know to be aggressive and impulsive in a delay-discounting task, selected the large/uncertain reward significantly less than vehicle-treated control rats, showing that testosterone increases aversion to uncertainty (Wallin, et al., 2015). However, these tasks tested impulsive choice which has a decision-making component. Impulsive choice as mentioned earlier (chapter 1) refers to one of the two behavioral manifestations of impulsivity. The substantial methodological differences between these studies and mine, make difficult to discuss my results in reference to these studies.

When looking at animal's response to changes in the probability of reward, both groups (control and subjugated) continue lever pressing the active lever regardless of the probability presented. Meanwhile, when a separate set of animals was tested in the same context but a fixed non-signal delay of 60 s was presented, subjugated animals immediately decrease the lever pressing response (experiment 2.3) showing an opposite response compared to controls. One possibility that could explain the contrary responses of

subjugate animals in both tasks could be related to the unexpected change in the contingency between response-reward. Because in the probability task, animals experience various different probabilities across 4 days of testing, and in the 60 s delay experiment animals have not previously experience any delay in the reward, it is possible that the difference between the results could be related to the unfamiliarity of the change. To evaluate this possibility, I compared the overall lever presses response of animals that were exposed to the 12% probability the first day of testing with those that had the 50 or 100% probability (50 and 100% were combined to have enough animals on each group, control and subjugated). The two-way ANOVA (independent variable: group and probability) indicated that there were no significant effects of group [$F(1,18)= 2.3759$, $p>0.05$], probability tested the first day [$F(1,18)= 0.5643$, $p>0.05$] or significant interaction between group and probability [$F(1,18)= 1.0896$, $p>0.05$]. This analysis discards the possibility that the difference in the response in subjugated animals relates to the unexpected changes in the contingency. Overall, the data suggest that stress has a selective effect on delayed reward and not in changes in the effort required or probability to obtain a reward. This finding is very interesting as it relates the effects of early stress exposure to selective changes in the response to delay rewards, rewards expectations and emotional responses, which are associated with different neural substrates.

For these perseverance tasks, two separate sets of animals were used. The results in both tasks showed a significant effect of set in some parameters. In particular, it was observed that the second set of animals showed higher frequencies compared to the first set of animals. It is important to mention that this difference between sets was observed since the training phase (lever press acquisition), which indicates that these differences were not related to the testing phase. Additionally, since the protocols for training and

testing were the same for both sets, and both groups, control and subjugated, included animals from these sets, there are no significant reasons to think that differences between sets could influence the results obtained.

Probability and delay discounting are both described by hyperbolic functions (Rachlin, et al., 1991). This finding that temporal and probability discounting functions have the same mathematical form have raised the idea that there may be similarities in the underlying process. However, some studies have described differences between temporal and probability discounting that question the validity of a single-process (Green & Myerson, 2004). Although my studies did not test discounting, it is interesting to highlight the opposite results observed in delay and probability in the present experiments. I believe that my data suggest that there is something unique about delay in the context tested in experiment 2.3 that is driven this unique behavioral profile. This possibility will be further discussed in chapter 5.

CHAPTER 5: DISCUSSION

The goal of the present set of experiments was to determine whether animals exposed to social stress in early adolescence, who as adults present enhanced aggression evidenced by higher frequency and repetitive patters of attacks, and decreased latency of attacks during encounters (Delville et al., 1998, Wommack et al., 2003), are impulsive, particularly if they show enhanced impulsive action. Furthermore, since impulsivity and aggression in clinical populations have been related to a lack of perseverance (Bøen, et al., 2015), these animals were also tested for perseverance as part of the behavioral profiling.

Action inhibition, one of the manifestations of impulsive action, refers to the ability to withhold a response. This ability was tested with a Go-NoGo task in which animals learned to lever press in response to a light cue (Newman, et al., 1985). During testing, animals were presented with a combination of trials: Go trials where only a light cue was present, and NoGo trials where a light and a tone were present at the same time. During Go trials animals are expected to lever press for food pellet rewards, and during NoGo trials they are expected to withhold the response and not lever press to obtain the reward. Failure to inhibit the response during NoGo trials is regarded as a measure of impulsive action. It was observed that previously stressed animals were less likely to inhibit the response in NoGo trials than control animals. This was evidenced by fewer testing session with successful NoGo trials (i.e., NoGo trials in which animals did not lever press), more NoGo trials presented, and more NoGo cue presentations as a result of lever presses during NoGo trials that reset the presence of the light and the tone.

In previous studies, social stress in early adolescence has been associated with aspects of impulsivity. In particular, rats exposed to social stress and tested in an open field with a novel object and in an elevated plus maze were characterized as risk takers and

novelty seekers (Toledo-Rodriguez & Sandi, 2011). Nevertheless, the relation between stress in adolescence and action inhibition has not been addressed. As such, data from this experiment extend these previous observations and are the first to show an effect of social stress in early adolescence on impulsive action.

Because the failure to inhibit the response in the Go-NoGo task could be related to a lack of extinction (Izquierdo & Jentsch, 2012), the specificity of the effect of early stress exposure on action inhibition was tested with an extinction experiment and a delayed in reward test. In the extinction experiment both groups, control and subjugated, showed decreased frequency of lever pressing the first day of reward omission. Thus, early social stress exposure does not have an effect in the acquisition of an extinction task, supporting the hypothesis that the differences between groups in the Go-NoGo task are due to an incapacity to withhold the response. Testing these animals in a complete omission of the reward could be seen as an extreme procedure, therefore I decided to use a less extreme procedure to continue testing the specificity of the effect on action inhibition. In the second control experiment, animals were trained to lever press in the absence of light cues for an immediate food pellet reward. During testing, a 60 seconds delay in the delivery of the reward was introduced. In this case, there was a difference between groups, and subjugated animals showed accelerated inhibition response as compared to controls, and lost the interest in the lever associated with the reward within the first 10 min on the first day of testing. Thus, early social stress exposure has an effect when the reward is delayed, but it is opposite to the response observed in the Go-NoGo task. These two control experiments showed that the differences between groups in the Go-NoGo task is not related to a lack of extinction. Instead when the reward was omitted subjugated animals showed a faster decrease of the response. Additionally, when the reward was delayed there was an opposite

response compared to the one observed in the Go-NoGo, and subjugated animals decreased faster the response. These two experiments support the hypothesis that the differences between groups in the Go-NoGo task are due to an incapacity to withhold the response.

These first experiments support the possibility that subjugated animals are impulsive, explaining their lack of aggressive control, in particular the observed enhanced attack frequencies during encounters (Delville et al., 1998, Wommack et al., 2003). A second aspect of impulsive action is an incapacity to delay a response (i.e. waiting impulsivity). Waiting impulsivity refers to the ability to withhold a response while waiting for the opportunity to respond to obtain a reward. Studies on aggression use attack latencies as a determining factor, as animals consider aggressive typically attack faster. In the case of subjugated animals that showed enhanced aggression in adulthood, they also showed decreased latency of attacks and bites (Delville et al., 1998, Wommack et al., 2003), which could be also interpreted as an incapacity to delay an action.

Animal's ability to wait to respond was tested in a modified version of the 5-Choice-Serial-Reaction-Time task (5-CSRTT) (Robbins, 2002). In this task, animals learned to respond to the presentation of a main house-light by nose-poking in any of two, adjacent illuminated ports. During testing, variable delays were introduced between the illumination of the main house-light and the ports that determine the opportunity to respond to get the reward. Waiting impulsivity was determined by animals' ability to withhold the response and wait for the opportunity to nose-poke in the illuminated ports to obtain a reward. Contrary to predictions, subjugated animals showed a lower percentage of premature responses by the longest delay than their controls, indicating that these animals were more capable of delaying actions. These data suggest that early social stress exposure has an enhancing effect on the waiting component of impulsive action. To my knowledge,

such findings have never been reported in animal models of stress and impulsivity. These findings also suggest that the short attack latencies observed in subjugated animals as adults, are not associated with waiting impulsivity in this context.

To confirm the specificity of the effect of early socials tress exposure on the ability to wait for the opportunity to respond, I evaluated the animals in a control experiment with a similar protocol but the delay was presented after the response, therefore instead of waiting to respond, animals had to wait for the reward in a Variable Delay in Delivery of Reward task (VDDR). In this experiment, animals were trained in the same protocol as for the modified 5-CSRTT, but during testing variable delays were introduced in the delivery of the reward. In this case, control and subjugated animals equally increased the percentage of repetitive response (i.e., nose-pokes made while waiting for the reward). Additionally, with longer delays animals in both groups become less accurate, but did not change their omission rate. These results suggest that in this context, early social stress exposure does not affect the ability to wait for a reward. These data support the specific effect of stress on waiting impulsivity, and suggest that stress differentially affects waiting for the opportunity to respond and waiting to receive a reward.

The results from these two experiments are very interesting and suggest that stress exposure might not always have detrimental effects, and that it may cause deficits in one form of impulsivity but not others. Improvement after stress exposure has been observed in radial-arm maze, object location task, and Y-maze (Bowman, Zrull, & Luine, 2001; Bowman, Ferguson, & Luine, 2002; Beck & Luine, 2002; Wright & Conrad, 2005; Mclaughlin, Baran, Wright, & Conrad, 2006). Even though, these studies did not test impulsivity, it is worth mentioning that the enhancing effects of stress on behavioral responses have been observed in other tasks as well. Although, these enhancing effects

have been mostly observed in females, it has been suggested that housing condition and specificities of the tasks are critical variables for male models of stress that can influence the effects of stress manipulation on behavior (Beck & Luine, 2002; Wright & Conrad, 2005). The specificity of early stress exposure on impulsivity is not surprising as different forms of impulsivity are related to different brain mechanisms. This will be discussed later in relation to the possible brain mechanisms associated with the behavioral profile observed in subjugated animals.

Another unique result from this set of experiments is the opposite response of subjugated animals in the response to delay in the delivery of the reward. To the introduction of a 60 s delay in the delivery of the reward subjugated animals immediately decrease the frequency of lever pressing and lose the preference for the area near the lever associated with the reward. On the other hand, in the context of the VDDR task, subjugated animals did not differ from controls and seem to overall increase the frequency of nose-poking (e.g., increase percentage of errors and repetitive responses). These two opposite responses to the introduction of a delay in the delivery of the reward in subjugated animals might be related to selective effects of stress on different learning mechanisms, or to differences in the processing of signaled-rewards in these two different contexts. These two possibilities will be discussed later with regard to the predicted neural mechanisms associated with the behavioral changes observed in stressed animals.

Finally, the behavioral profiling of animals exposed to social stress in early adolescence included two tests of perseverance. In this case, animals were tested in the same context were the most dramatically differences between stress and control animals were observed. For this, animals were trained in the absence of light cues to lever press to obtain immediate food pellet rewards. During testing different lever presses requirements

or probabilities of reinforcement were presented. Perseverance tested under these conditions was not affected by early stress exposure, as control and subjugated animals did not differ in any measure, and continue to lever press in spite of the changes in effort or probability of reinforcement. The results of these experiments suggest that these animals do not show lack of perseverance when tested under these conditions. However, taking together this with the results observed when the reward was delayed, the data suggest that the effect on perseverance is selective to delay in the rewards and it is context-specific. This suggest that stress might be having selective effects on brains mechanisms associated with tolerance to delayed rewards.

Behavioral consequences of social stress exposure in early adolescence

Previous work in our lab and the results of the present studies have helped build a behavioral profile that shows the long-term effects of social stress exposure in early adolescence. The observed effects help to make predictions about possible neurochemical changes related to these effects and the proposition of a new model of aggression.

In particular, it has been observed that exposure to chronic social stress in early adolescence leads to specific short and long-term behavioral effects. The short-term effects include: avoidance and caution, increased submissive behaviors and inhibition of offensive aggression when tested with adult males (Bastida, et al., 2009; Wommack, et al., 2004, Delville, et at., 1998; Wommack, et al., 2003). When tested with younger males on the other hand, there is an acceleration of the maturation of agonistic behavior (Delville, et al., 1998; Wommack, et al., 2003). The long-term effects include: quick reinstatement of avoidance after a defeat (Ferris, Messenger & Sullivan, 2005), and enhanced aggression when tested with younger animals (Wommack, et al., 2003). While these animals avoid

unknown adults, they do not present generalized anxiety (Bastida, et al., 2009). The present studies, while including selective alterations in impulsivity, also show that animals are not completely impaired, as they can learn associative tasks. In particular, these animals present impaired action inhibition but enhanced waiting impulsivity, intolerance to context-specific delayed gratification, without changes in perseverance when tested with changes in effort or probabilities of reinforcement.

Brain mechanisms associated with the phenotype observed

Impulsivity being a multifeatured concept involved multiple neurochemical systems and neural pathways. In general, it has been observed that action inhibition involves the inferior frontal gyrus (IFG), orbitofrontal cortex (OFC), dorsomedial striatum (caudate-putamen), Globus pallidum (GP), subthalamic nuclei (STN), Thalamus, Nucleus accumbens core (NAc-core), Substantia Nigra (SNc), Ventral tegmental area (VTA), and Raphe nuclei. On the other hand, waiting impulsivity is related to infralimbic cortex (IL), ventrolateral prefrontal cortex (VPFC), anterior cingulate (ACC), hippocampus (HC), Septum, and Nucelus Accumbens core (NAc-c) and shell (NAc-s) (Figure 26) (Dalley, Everitt, & Robbins, 2011; Jentsch, et al., 2014; Dalley, et al., 2008; Pattij & Vanderschuren, 2008; Eagle & Baunez, 2010).

Exposure to stress increases DA, 5-HT and norepinephrine activity (Butts, Weinberg, Young & Phillips, 2011; Adell, Casanovas, & Artigas, 1997; Amat, Matus-Amat, Watkins, & Maier, 1998; Fujino, Yoshitake, Inoue, Ibii, Kehr, Ishida, Nohta, & Yamaguchi, 2002; Rossetti, Portas, Pani, Carboni, & Gessa, 1990). I think that this increase activity during stress exposure in early adolescence, is followed by a compensatory long-lasting inhibition of these system (Watt, et al., 2014; Tang, Lei, Sun, Liu, & Zhao, 2013),

in specific areas associated with the different forms impulse control. This suggests, that early stress exposure might have overlapping and site-specific effects in these systems.

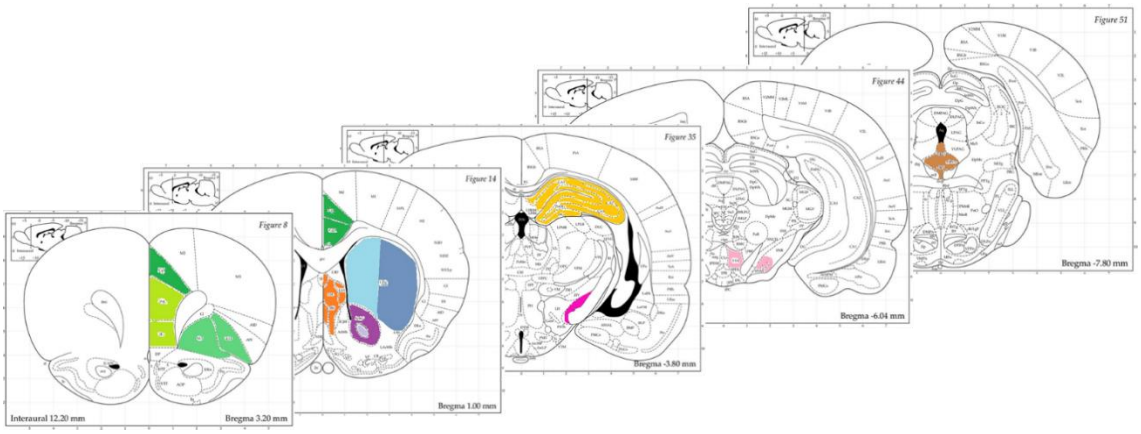


Figure 26. Representation of structures involved in control of impulsivity. Adapted from: The rat brain in stereotaxic coordinates: hard cover edition. Access Online via Elsevier, 2006. In green: IFG (inferior frontal gyrus), OFC (orbitofrontal cortex), ACC (anterior cingulate), IL (infralimbic cortex). In orange: Medial and lateral septum. In light purple: NAc-core (Nucleus accumbens core), dark purple: NAc-s (Nucelus Accumbens shell). In blue: Striatum. In fuchsia: STN (subthalamic nuclei). In yellow: HC (Hippocampus). In pink: SNc (Substantia Nigra) and VTA (Ventral tegmental area). In brown: Raphe nuclei.

STRESS, DOPAMINE AND ACTION INHIBITION.

In relation to dopamine and action inhibition, I think that the activation of DA by stress exposure, is followed by a compensatory inhibition in specific structures related to the control of action inhibition. During adolescence there is an increase in DA activity in striatal, limbic and cortical structures, and an increase in D1 and D2 receptors density in subcortical structures such as striatum and NAc (Wahlstrom, White, & Luciana, 2010). I believe that, chronic exposure to early social stress, which causes increase in DA release in the PFC (Butts, et al., 2011), leads to alteration in the development of this system,

causing in adulthood down-regulation of the mesocortical system evidence by decrease in dopaminergic activity in the PFC (Watt, et al., 2014), and possible in striatal and limbic structures. Typically, DA activity in the PFC, from VTA innervations, inhibits glutamatergic input to the striatum, thus reducing excitatory input to these structures (Watt, Weber, Davies, & Foster, 2017). Perhaps decrease DA activity in cortical, striatal and limbic structures, with an imbalance in the direct and indirect cortico-striatal pathways involving the STN and its dopaminergic input from substantia nigra, in addition to possible upregulation of D1 and/or D2 receptors in the striatum, may generate alterations in the inhibition of motor responses (Morein-Zamir & Robbins, 2015; Leisman, Melillo, & Carrick, 2013; Cragg, Baufreton, Xue, Bolam, & Bevan, 2004) (Figure 27). This could explain the enhancing effects that dopamine augmenting drugs have on impulsive action (de Wit, et al., 2002; van Gaalen, Brueggeman, et al., 2006; Hayton, et al., 2012).

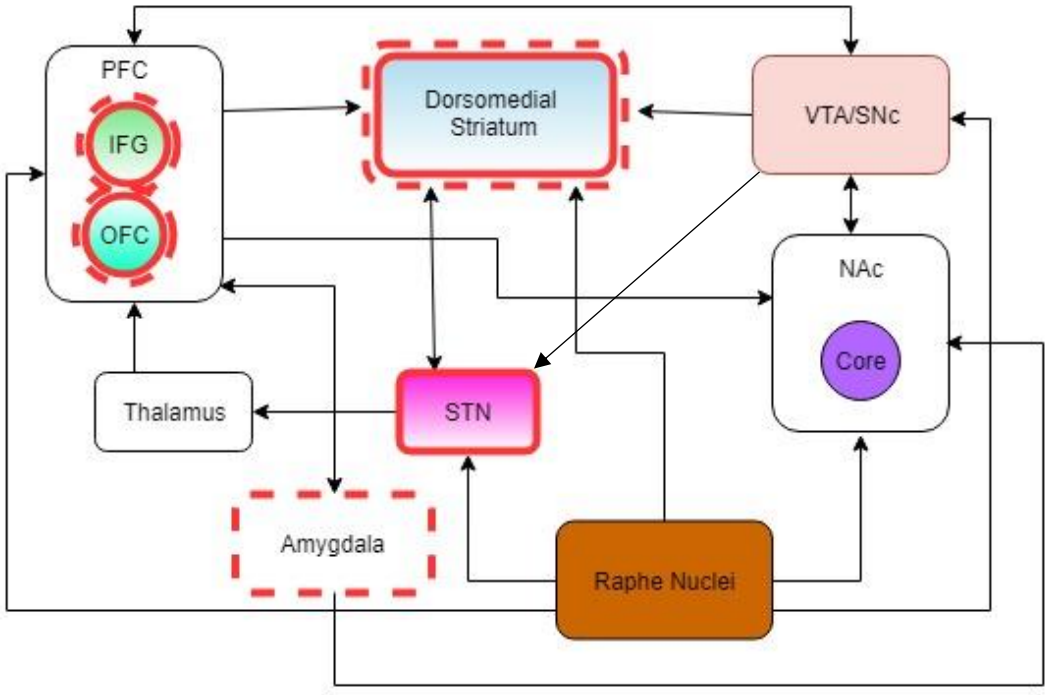


Figure 27. Schematic representation of the neural system controlling action inhibition and proposed changes in DA and 5-HT in subjugated animals. Highlighted structures represent the hypothesized alterations in animals exposed to stress in early adolescence. Solid red lines represent dopamine downregulation and dashed lines represent downregulation of serotonin. PFC: prefrontal cortex; IFG: inferior frontal gyrus, OFC: orbitofrontal cortex, STN: subthalamic nuclei; SNc: substantia nigra; VTA: ventral tegmental area; NAc: nucleus accumbens core.

STRESS, DOPAMINE, AND WAITING IMPULSIVITY

Some studies have shown the role of DA depletion, or systemic administration of DA receptor agents in waiting impulsivity, however because some of the manipulations used affect the attention component of the tasks by decreasing the accuracy (Robbins, 2002), the specific role of dopamine in waiting impulsivity is not clear. Based on the available literature, in relation to dopamine and waiting, I think that the compensatory inhibition followed chronic activation of DA by stress exposure, might decrease the DA

activity in the Nucleus Accumbens (Cole & Robbins, 1989), promoting reduce premature responses. This is supported by studies showing that d-amphetamine administration increases premature responses, while DA antagonists decrease these responses (van Gaalen, Brueggeman, et al., 2006) (Figure 28).

STRESS, SEROTONIN, AND ACTION INHIBITION

In relation to action inhibition, an alternative possibility to DA compensatory inhibition, is that the compensatory serotonergic inhibition during adulthood, is related to the deficits observed. As stated in the introduction, several studies have shown that central serotonin depletion impairs the performance in Go-NoGo tasks (Harrison, Everitt, & Robbins, 1999). I predict decrease 5-HT transmission in medial prefrontal cortex, medial striatum, and amygdala, which correspond to structures involved in the control of action inhibition (Figure 27) (Masaki, et al., 2006; Aron & Poldrack, 2005)

STRESS, SEROTONIN, AND DELAYED REWARD

An interesting effect was observed in relation to waiting for a reward. When a 60 seconds delay in the delivery of the reward was introduced in a setting where no light-cues signaled the opportunity to respond, previously stressed animals immediately decrease the lever pressing response and lost the preference for the area near the lever associated with the rewards. When variable delays were introduced in the VDDR task in the 5-CSRT boxes, previously stressed animals did not differ from control and continue nose-poking regardless of the delay presented. These opposite responses suggest that there is something unique about delayed gratification that is being selectively affected by early stress exposure.

Exposure to stress increases 5-HT release in numerous areas including amygdala, frontal cortex, hypothalamus, and raphe nuclei (Adell, et al., 1997; Amat, et al., 1998; Fujino, et al., 2002). This has also been observed in juvenile hamsters at P45 right after stress exposure, where subjugated animals showed an increase in serotonergic density within the anterior hypothalamus and lateral septum, suggesting an enhanced capacity to release 5-HT compared to controls (Delville, et al., 1998). Therefore, I predict that chronic stress exposure in early adolescence activates the release of 5-HT, but this sustained chronic activation, is followed by a compensatory inhibition during adulthood in specific structures related to the control of delay reward.

In particular, I predict that subjugated animals after chronic stress exposure, have a long-term compensatory mechanism resulting in lower levels of serotonin in the dorsal raphe that disturbs their capacity to wait for a delayed reward, possibly acting through its projections to NAc and PFC (Figure 28) (Miyazaki, et al., 2011a; 2011b, 2012; Miyazaki, Miyazaki & Matsumoto, 2004; Fonseca, et al., 2015). This is supported by studies that have shown that the serotonergic dorsal raphe (DR) neurons are involved in the promotion of waiting for a reward. Thus, a high expectation or confidence in future rewards and unpredictability of the waiting time, make more difficult to reject the possibility that the reward may still come, and are necessary for serotonin neural activation to promote waiting (Miyazaki, et al., 2018). However, this does not explain why subjugated animals decrease the response in the 60 s delay reward task, but not when tested in the VDDR context. For these context- specific responses in delay rewards in relation to serotonin I have two hypotheses:

1. Reduce response due to decrease processing of reward-related signals. When tested in the VDDR in the 5-CSRT boxes, animals are trained to respond

based on visually external cues that signal the opportunity to make a nose-poke and receive future rewards. On the other hand, when tested in the 60 s task, the conditioning chambers were not equipped with light cues, and the lever that signals the opportunity to respond was always present. Thus, this context did not provide discrete external cues, and the animals needed to rely exclusively on the context to adjust their confidence in future rewards. Perhaps, in subjugated animals there is less activation of the glutamate and 5-HT neurons from the DR that project to the VTA, and promote waiting for rewards by the evaluation of reward-related signals (Luo, Zhou, & Liu, 2015; McDevitt, et al., 2014). Thus, because there might be a decrease activity in the DR-VTA connection related to the processing of reward-related signals and decrease serotonin in the dorsal raphe that promotes waiting for reward, in the absence of discrete cues, this overall decreased activity in the DR is not enough to promote waiting for the delayed reward, therefore subjugated animals decrease the lever pressing behavior (Figure 28).

2. Reduce response due to alterations in contextual learning mechanisms. The relevance of cues and context in associative learning has been extensively study in fear conditioning tasks. Following pairing of an unconditional stimulus (US), such as a foot-shock, with a conditional stimulus (CS), a particular context and/or a cue, animals will perform a conditional response (CR) such as freezing in the presence of the CS (Davis, 1992; Fendt & Fanselow, 1999). In contextual fear conditioning, an animal is placed in a novel environment, and presented with an aversive stimulus, and then removed from this context. When animals are returned to the same environment, generally demonstrate freezing response

if they remember and associated that environment with the aversive stimulus. On the other hand, cued fear conditioning is similar to contextual conditioning, with one difference, the presence of a CS in the context. In order to separate context from cue conditioning, animals can be pre-expose to the context without the presentation of the US (Fendt & Fanselow, 1999; Curzon, Rustay, & Browman, 2009).

Cued fear conditioning has been related to the dopaminergic system and basolateral amygdala (Fadok, et al., 2009; Pezze, & Feldon, 2004; Maren, 2001). On the other hand, context fear conditioning has been related to Hippocampus (Phillips & LeDoux, 1992; Anagnostaras, et al., 2001; Selden, et al., 1991; Kim, & Jung, 2005; Chang & Liang, 2017). Moreover, it has been observed that inhibition of the ascending serotonergic from the medial raphe neurons to the hippocampus or activation of 5-HT1A receptors in the dorsal hippocampus itself, decrease contextual fear conditioning (Almada, Borelli, Albrechet-Souza, & Brandão, 2009), suggesting a possible role of serotonin in the hippocampus in the modulation of contextual fear conditioning.

Even though this evidence comes from fear conditioning, it is useful to make predictions about the selective effects that early stress exposure can have in different learning mechanisms. The fact that subjugated animals continue responding in the same way that control animals when the delay was presented in the VDDR in 5-CSRT boxes, where a light cue signals the possibility to respond for a reward, suggests that early stress exposure does not affect the systems involved in cue-associative learning. However, the decrease response in the 60 s delay reward task (as previously stated a contextual learning), is

possibly related to some detrimental effect on the contextual learning mechanisms, probably in the hippocampus or in the afferent serotonergic projections from medial and dorsal raphe, and also in the projections to the septum (Figure 28). It is possible that in this contextual learning, in subjugated animals once the relation between response and reward is modified by introducing a 60 seconds delay, the context representation in relation to the reward, is not strong enough to sustain the behavior causing a decrease in the response.

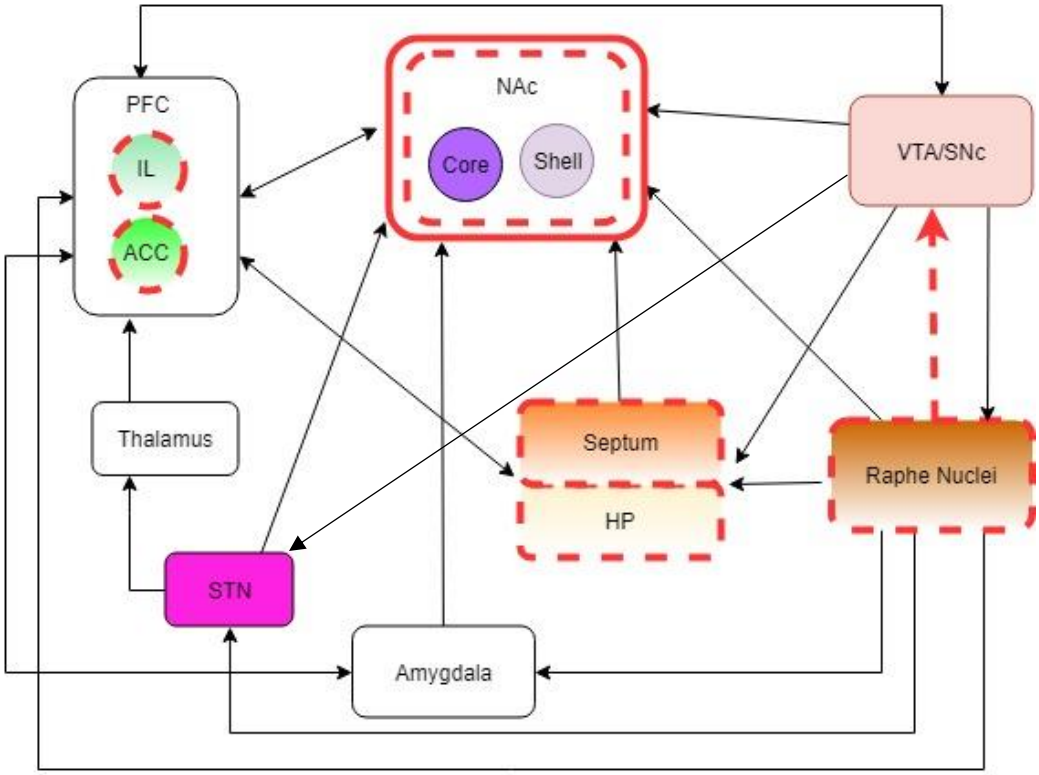


Figure 28. Schematic representation of the neural system controlling waiting and proposed changes in DA and 5-HT in subjugated animals. Highlighted structures represent the hypothesized alterations in animals exposed to stress in early adolescence. Dashed red lines represent downregulation of serotonin, and solid lines represent dopamine downregulation. PFC: prefrontal cortex; IL: infralimbic cortex; ACC: anterior cyngulate; NAc: Nucleus accumbens; HP: hippocampus; SNc/VTA: Substantia nigra/Ventral tegmental area.

STRESS, SEROTONIN, AND WAITING IMPULSIVITY

In relation to waiting impulsivity and based on the literature, it would be expected an opposite effect of stress on serotonin from the one already discussed. Since, serotonergic projections from the dorsal raphe innervate the NAc, caudate-putamen, globus pallidus, substantia nigra and the amygdala (Harrison, Everitt, & Robbins, 1997a), in this case stress would cause and increase 5-HT transmission in these structures in adulthood to promote

enhance waiting impulsivity. In particular, the NAc and its connection with hippocampus and prefrontal cortex that have been broadly involved in the control of waiting impulsivity (Dalley, et al., 2011). This alternative hypothesis could be explained by stress in early adolescence having an epigenetic effect on the 5-HT1B receptors that are expressed in adulthood in structures that have been related to impulsive action. In particular, it would be expected an increase of these receptors in the cortex, striatum, NAc, subthalamic nuclei, hippocampus, and substantia nigra (Nautiyal, et al., 2015). However, this upregulation of 5-HT in waiting impulsivity, does not fit with the propose changes in 5-HT based on the behavioral response to delayed reward. Therefore, I think that the effect of stress on waiting impulsivity is probably modulated by the alteration in the dopaminergic system.

Possibilities for rescuing the behavior

Considering the behavioral consequences of chronic stress exposure in early adolescence and the possible brain mechanisms associated, the next point to consider is the possibilities for rescuing the behavior in adulthood. As mentioned earlier, serotonin has been widely involved with impulsivity, therefore, one option would be the use of serotonin reuptake inhibitors such as Fluoxetine (Prozac). Systemic administration of fluoxetine in adult hamsters inhibits offensive aggression (Delville, Mansour, & Ferris 1996; Ferris et al., 1997). Therefore, it would be possible that this treatment could have an effect on impulsivity as well. However, fluoxetine may be having an effect on aggression through another mechanism not associated with impulsivity. Another aspect to consider would be when to administer fluoxetine, either in adolescence or adulthood. During adolescence the serotonergic system is undergoing maturation, which suggests that administration of fluoxetine during this time could have different effects than during adulthood. In fact, it

has been observed that in juveniles, the low dose of fluoxetine has the opposite effect than in adults, and activates agonistic behaviors (frequency of attacks, attacks per bout, and contact time). Unlike adults, the high dose resulted in only a partial inhibition of agonistic behavior (Taravosh-Lahn, Bastida, & Delville, 2006). Therefore, the evidence suggests that the use of fluoxetine as a possibility for rescuing the behavior would have to be during adulthood.

Administration of fluoxetine in adulthood could be a possibility for rescuing the behavior after early stress exposure, however this intervention may or may not work, because as explained earlier, the effects of early social stress exposure may be very localized, therefore any possible intervention would need to be site and receptor specific. More studies need to be done to evaluate the effect of fluoxetine on the behaviors observed in these animals.

Toward a new model of aggression

Chronic social stress exposure in early adolescence causes a variety of behavioral changes including enhance aggression, decrease action inhibition, increase waiting impulsivity, and context-dependent aversion to delay gratification. These are complex behaviors that involved multiple brain mechanisms and multiple neurotransmitters. My work has helped build the behavioral profile of adult animals exposed to chronic social stress in early adolescence, and allows the propositions of brain mechanisms associated with these behaviors observed.

The notion of impulsive-aggression originated from studies correlating decreased serotonergic activity with suicide, personality disorders, self-report hostility and aggression, and problematic behaviors in criminal population (Coccaro, 1989). However,

this impulsive-aggression profile did not identify subtypes of impulsivity or considered different forms of aggression. Work from our lab and others, have helped to better define the relation between aggression and impulsivity, by looking at common features observed in the different types of aggression in animals and humans. In particular, proactive aggression in humans shows parallels with injurious aggression in animals, since it is associated with inhibited emotional reactivity, and goal directed behaviors. On the other hand, reactive aggression in humans show parallels with offensive aggression in animals, since it is associated with enhanced emotional reactivity and impulsivity (Cervantes & Delville, 2007; David, et al., 2004; Kockler, et al., 2006; Haller, et al., 2001).

Different authors associating aggression with impulsivity, have observed different patterns of impulsive behaviors associated with aggression (Table 6). For example, it has been observed that testosterone administration enhances aggression (Wood, et al., 2013), decreases impulsive choice, and enhance risk taking (Wood, et al., 2013), without affecting performance in a Go-NoGo task compared to vehicle-treated controls (Cooper, et al., 2014). On the other hand, inherently aggressive hamsters show impulsive choice on a delay discounting task (Cervantes & Delville, 2007; Cervantes & Delville, 2009). This ambiguous relation between aggressive and impulsive behaviors suggests that perhaps there are multiple types of impulsive-aggression profiles, related to different brain mechanisms.

Aggression		Impulsivity and other conditioned behaviors		Reference
Testosterone (Long-Evans)	+	-	Impulsive choice (Delay)	Wood, et al., 2013; Cooper, et al., 2014; Wallin, et al., 2015, 2018
		+	Risk taking	
		=	Action inhibition (Go/NoGo)	
		+	Probability discounting	
		-	Effort discounting	
High-Agg (Hamsters)	+	+	Impulsive choice (Delay)	Cervantes & Delville, 2007; Cervantes & Delville, 2009; David, et al., 2004
		+	Lever pressing delay in reward	
Roman-Low-Avoidance	+	+	Action inhibition (5-CSRT)	Coppens et al., 2012; Moreno et al., 2010
		-	Impulsive choice (Delay)	
Roman-High-Avoidance (RHA)	-	+	Impulsive choice (Delay)	
		-	Action inhibition (5-CSRT)	
		+	Lever presses in VI-15	
RHA- after stress	+	-	Lever presses in VI-15	Coppens et al., 2012
Hamsters-after stress in adolescence	+	-	Action inhibition (Go/NoGo)	Gonzalez, et al., 2017
		+	Action inhibition (modified 5-CSRTT)	
		-/=	Lever pressing delay in reward	
		=	Perseverance (Effort and Probability)	
		=	Extinction	
Wild-type Groningen (WTG) rats	+	+	Lever presses in VI-15	Coppens, et al., 2014
		=	Differential-reinforcement-of-low-rates	
		=	Extinction	

Table 6. Summary of studies showing relation of aggression with impulsivity and other conditioned behaviors. High-Agg: Inherently high aggression animals. + : increase; - : decrease; = : no difference with control or low aggression animals.

Maybe we should look at aggression as part of a complex personality construct involving different forms of aggression, different forms of impulsivity, different forms of emotional reactivity, and different forms of perseverance. We propose the redefinition of the concept of aggression, to a multidimensional construct that mediates personality (Figure 29). It is important to consider the existence of multiple impulsive-aggressive profiles that emphasize the fact that because an individual show enhance offensive aggression and reduce impulse control in one form of impulsivity, it does not mean that

this individual has deficits in impulse control in all or other forms of impulsivity. Even more, it is possible that this individual shows an enhance impulse control in another form of impulsivity.

I believe it is very important to reconsider the broad aggressive/impulsive profile used to characterize behavioral alterations in humans and animals, and recognize the existence of multiple of these profiles with the different features associated with impulsive behaviors, and emotional reactivity. This is very relevant to consider specially in the study of possible causes and interventions to treat these behavioral alterations. It is important to recognize that the underling structures, neurotransmitters, receptors, and pathways involved in the aggressive/impulsive profiles are different, therefore the targets for prevention and interventions need to be different.

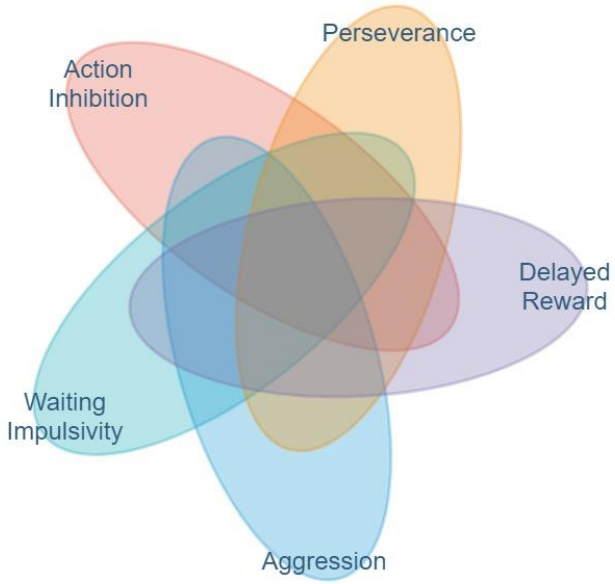


Figure 29. Proposed redefined model of aggression. Aggression should be redefined as a multidimensional construct that mediates personality. This personality construct should include different forms of aggression, impulsivity, emotional reactivity and perseverance. Different combination of behaviors leads to multiple aggressive-impulsive profiles.

By considering multiple impulsive/aggressive profiles, we can start explaining why male hamsters after social stress exposure in adolescence become aggressive adults. The results from my studies show that these animals show increased number of attacks toward intruders possible because they have decreased ability to withhold the offensive response and continue attacking. However, the enhanced ability to wait to respond observed in the waiting task with a modified 5-CSRTT, does not explain why these animals are faster are performing attacks. The differential response to delayed reward suggests that these animals may have some emotional response that cannot control and is driving their behavior. It is necessary to perform more studies to clarify these possibilities. However, these effects could be explained by a long-lasting down regulation of serotonin availability in areas common to aggression, impulsivity, and emotional reactivity, such as the lateral septum (David, et al., 2004; Delville, De Vries, & Ferris, 2000), which merges inputs from the hippocampus with inputs from the medial amygdala, and projects to the ventrolateral hypothalamus (Ferris, Gold, De Vries, & Potegal, 1990; Gomez & Newman, 1992).

TRANSLATIONAL RELEVANCE

In humans, the relation between aggression and impulsivity in clinical populations has shown the same ambiguity from the perception of a unique impulsive-aggressive profile. For example, Borderline personality disorder (BPD) and Bipolar Disorder (BP) have been characterized by aggression and impulsivity. BPD has been related to enhanced impulsive action in a delay discounting task (Lawrence, et al., 2010; Barker, Romaniuk, Cardinal, Pope, Nicol, & Hall, 2015), but the opposite has also been observed (Dougherty, et al., 1999). Additionally, BPD has been related to impulsive action in a Go-NoGo task (Rentrop, et al., 2008), however, other studies have not observed alteration in impulsive

action in a Stop-Signal Task (SST) (Barker, et al., 2015). On the other hand, BP disorder has been related to enhance impulsive choice (Ahn, et al., 2011) and impulsive action in a Go-NoGo task and SST (Fleck, et al., 2011; Strakowski, et al., 2010). However, in another study BP patients did not differ from controls in a risk-taking task (Reddy, Lee, Davis, Altshuler, Glahn, Miklowitz, & Green, 2014). Nevertheless, one difference between BPD and Bipolar seems to be perseverance. Some studies have shown differences in perseverance between these psychopathologies (Bøen, et al., 2015), while some others have not (Shafiee-Kandjani, et al., 2017). These studies suggest that perhaps the characterization of these disorders based in aggressive and impulsive behaviors, should incorporate emotional components, and other behaviors such as perseverance, that may allow a better understanding of these multiple impulsive/aggression profiles in clinical populations. By considering the possibility of multiple profiles and their specific underling brain mechanisms, the treatment of these disorders could be improved by targeting specific alterations in the neural systems associated with the multidimensional behavioral alterations.

By recognizing a multidimensional concept of aggression, using my experiments we could possibly translate my results to humans, and predict that exposure to stress in early adolescence will be related to specific clinical disorders involving various forms of impulsivity, such as action inhibition and waiting, and specific behavioral deficits, possibly in the inhibition of prepotent motor responses and context-dependent aversion to delay gratification. These profiles will involve specific neural mechanisms as previously exposed.

Future directions

In the behavioral profile of male hamsters exposed to chronic social stress in early adolescence we have not study impulsive choice. I think, the next step will be to evaluate these animals in impulsive choice tasks including: delay discounting and risk taking. Additionally, even though I tested action inhibition in a Go-NoGo task, it would be interesting to test these animals in another task for action inhibition like the Stop-Signal-Task (SST) and evaluate if the decrease ability to withhold a response is also present in this task.

The biggest effect observed in the animals exposed to stress in early adolescence was the response to delayed rewards. I think that the context-specific aversion to delay rewards needs to be further study, especially in terms of a possible emotional component involved in this response. All the tasks used in my experiments included a training protocol in which animals learn to make one response to obtain 1 food pellet reward, in other words I used a fixed-ratio-1 (FR-1) program of reinforcement. The use of continuous reinforcement produces a stronger expectancy of the reward, and a stronger frustration when the reward is not presented (Amsel & Roussel, 1952; Amsel, 1992). I propose two ways to evaluate the frustration response when the reward is delayed. The first one, includes training the animals in a partial reinforcement schedule, in which during training the lever press response is not always reinforced. That way, based on frustration theory, with sufficient training partial reinforcement results in learning to make the instrumental response when the subject expects nonreward. Once the response has become conditioned to the expectation of nonreward responding persist when the reward is omitted (Amsel & Roussel, 1952; Amsel, 1992). I believe that the same effect could be observed when the reward is delayed by 60 seconds. Partial reinforcement seems to decrease the frustration in

the face of non-immediate reward and promote persistence. I think that by training the animals with partial reinforcement, the response to the introduction of the 60 seconds delay in the delivery of the reward can evaluate the frustration response in subjugated animals.

The second way to evaluate the frustration component could be using the successive contrast protocol. The Successive Negative Contrast (SNC) effects refers to a decrease in the consummatory behavior when animals experience unsignaled decline in the quantity or palatability of a familiar food source. The decrease in the behavior falls below of control animals that have only experienced the lesser reward, and returns to baseline levels after 4-5 days (Mitchell & Flaherty, 2005). Exposing rats to an unexpected 4% sucrose after receiving 32% sucrose for 12 days, showed a massive cortical activation observed with c-fos, especially in the orbital prefrontal cortex, infralimbic, prelimbic and cingulate cortex. Additionally, c-fos activity was also high in hippocampus, Nucleus Accumbens-shell, Globus pallidum (major output pathway of the dorsal striatum), BST and septum (Pecoraro & Dallman, 2005). I think it would be interesting to evaluate hamsters exposed to stress in early adolescence to a successive contrast protocol to evaluate their behavioral response, and also c-fos activation.

Finally, I think it is very important to do address the long-term neural changes related to chronic stress exposure in early adolescence. First, I believe it is important to evaluate content and turn-over rates of serotonin and dopamine in the brain areas proposed in my work in relation to the aggression/impulsive profile observed. This could be done with High-Performance-Liquid-Chromatography (HPLC) comparison in adulthood, but also with developmental comparison between control and subjugated animals. Additionally, immunohistochemistry can be done to evaluate receptors (5-HT1B, 5-HTD1,

5-HTD2, D1 and D2) and serotonin and dopamine innervation in the areas proposed in this work.

Finally, optogenetics and knock-out animals could be used to further study the role of these receptors and areas in the long-term effects of stress in early adolescence in adult aggressive and impulsive behaviors.

REFERENCES

Accorsi, P., Carloni,E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C., & Seren, E. (2008) Cortisol determination in hair and faeces from domestic cats and dogs. *General and Comparative Endocrinology*, 155(2), 398-402.

Adam, A., & Poldrack, R. (2005) The cognitive neuroscience of response inhibition: relevance for genetic research in attention-deficit/hyperactivity disorder. *Biological Psychiatry*, 57(11), 1285-1292.

Adams, D. B. (2006) Brain mechanisms of aggressive behavior: An updated review. *Neuroscience & Biobehavioral Reviews*, 30, 304–318.

Adams, L., Biglieri, E., & Bern, H. (1965) The adrenal cortex and its hormones in the california ground squirrel, *Spermophilus Beecheyi*. *Journal of Mammalogy*, 46(3), 446-450.

Adell, A., Casanovas, J., & Artigas, F. (1997) Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. *Neuropharmacology*, 36, 735-741.

Agrawal, A., Jaggi, A., & Singh, N. (2011) Pharmacological investigations on adaptation in rats subjected to cold water immersion stress. *Physiology & Behavior*, 103(3–4), 321-329.

Ahima, R., & Harlan, R. (1990) Charting of Type II glucocorticoid receptor-like immunoreactivity in the rat central nervous system. *Neuroscience*, 39, 579–604.

Ahima, R., Krozowski, Z., & Harlan, R. (1991) Type I corticosteroid receptor-like immunoreactivity in the rat CNS: Distribution and regulation by corticosteroids. *Journal of Comparative Neurology*, 313, 522–538.

Ahn, W., Rass, O., Fridberg, D., Bishara, A., Forsyth, J., Breier, A., Busemeyer, J., Hetrick, W., Bolbecker, A., & O'Donnell, B. (2011) Temporal discounting of rewards in patients with bipolar disorder and schizophrenia. *Journal of Abnormal Psychology*, 120(4), 911–921

Ainslie, G. (1975) Specious reward: A behavioral theory of impulsiveness and impulse control. *Psychological Bulletin*, 82(4), 463-496.

Albers, H., Yogev, L., Todd, R., & Goldman, B. (1985) Adrenal corticoids in hamsters: Role in circadian timing. *American Journal of Physiology*, 248(4 Pt 2), 434-438.

Alexis, M., Stylianopoulou, F., Kitraki, E., & Sekeris, C. (1983) The distribution and properties of the glucocorticoid receptor from rat brain and pituitary. *The journal of biological chemistry*, 258(8), 4710-4714.

Almada, R., Borelli, K., Albrechet-Souza, L., & Brandão, M. (2009) Serotonergic mechanisms of the median raphe nucleus–dorsal hippocampus in conditioned fear:

Output circuit involves the prefrontal cortex and amygdala. *Behavioural Brain Research,* 203 (2), 279-287.

Amat, J., Matus-Amat, P., Watkins, L., & Maier, S. (1998) Escapable and inescapable stress differentially and selectively alter extracellular levels of 5-HT in the ventral hippocampus and dorsal periaqueductal gray of the rat. *Brain Res.,* 797, 12-22.

Amsel, A. & Roussel, J. (1952) Motivational properties of frustration: I. Effect on a running response of the addition of frustration to the motivational complex. *Journal of Experimental Psychology: General,* 43(5), 363-368.

Amsel, A. (1992) Frustration theory: Many years later. *Psychological Bulletin,* 112(3), 396-399.

Amstislavskaya, T., Bulygina, V.V., Tikhonova, M.A., & Maslova, L.N. (2013) Social isolation during peri-adolescence or adulthood: effects on sexual motivation, testosterone and corticosterone response under conditions of sexual arousal in male rats. *Chin J Physiol*, 56(1), 36-43.

Anagnostaras, S., Gale, G., & Fanselow, M. (2001) Hippocampus and contextual fear conditioning: Recent controversies and advances. *HIPPOCAMPUS*, 11, 8 –17.

Apter, D., Pakarinen, A., Hammond, G., & Vihko, R. (1979) Adrenocortical function in puberty serum ACTH, cortisol and dehydroepiandrosterone in girls and boys. *Acta Paediatrica*, 68, 599–604.

Armario, A., Castellanos, J., & Balasch, J. (1984) Adaptation of anterior pituitary hormones to chronic noise stress in male rats. *Behavioral and Neural Biology*, 41(1), 71-76.

Aron, A., & Poldrack, R. (2006) Cortical and subcortical contributions to stop signal response inhibition: Role of the subthalamic nucleus. *The Journal of Neuroscience*, 26(9), 2424 –2433.

Baarendse, P., Counotte, D., O'Donnell, P., & Vanderschuren, L. (2013) Early social experience is critical for the development of cognitive control and dopamine modulation of prefrontal cortex function. *Neuropsychopharmacology*, 38(8), 1485–1494

Baenninger, L. (1967) Comparison on behavioural development in socially isolated and grouped rats. *Animal Behaviour*, 15, 312-323.

Báez, M., Siriczman,I, & Volosin, M. (1996) Corticosterone is involved in foot shock-induced inactivity in rats. *Physiology & Behavior*, 60(3), 795-801.

Bali, A., & Jaggi, A. (2015) Electric foot shock stress adaptation: Does it exist or not? *Life Sciences*, 130, 97-102.

Bari A., Dalley J., Robbins T. (2008) The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nature Protocols*, 3, 759–767.

Bari, A., & Robbins, T. (2013) Inhibition and impulsivity: Behavioral and neural basis of response control. *Progress in Neurobiology*, 108, 44–79.

Barker, V., Romaniuk, L., Cardinal, R., Pope, M., Nicol, K., & Hall, J. (2015) Impulsivity in borderline personality disorder. *Psychological Medicine*, 45(9), 1955-1964.

Barrett, A., & Stockham, M. (1963) The effect of housing conditions and simple experimental procedures upon the corticosterone level in the plasma of rats. *Journal of Endocrinology*, 26, 97-105.

Barton, B., Rahn, A., Feist, G., Bollig, H., & Schreck, C. (1998) Physiological stress responses of the freshwater chondrostean paddlefish (*Polyodon Spathula*) to acute physical disturbances. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 120(2), 355–363.

Bastida, C., Puga, F., & Delville, Y. (2009) Risk assessment and avoidance in juvenile golden hamsters exposed to repeated stress. *Hormones and Behavior*, 55(1), 158-162.

Bastida, C., Puga, F., Gonzalez-Lima, F., Jennings, K., Wommack, J., & Delville, Y. (2015) Chronic social stress in puberty alters appetitive male sexual behavior and neural metabolic activity. *Hormones and Behavior*, 66(2), 220–227.

Bateman, A., Singh, A., Kral, T., & Solomon, S. (1989) The Immune-Hypothalamic-Pituitary-Adrenal Axis. *Endocrine Reviews*, 10(1), 92-112.

Beck, K., & Luine, V. (2002) Sex differences in behavioral and neurochemical profiles after chronic stress: Role of housing conditions. *Physiology & Behavior*, 75, 661-673.

Bercovitch, F., & Clarke, S. (1995) Dominance rank, cortisol concentrations, and reproductive maturation in male rhesus macaques. *Physiology & Behavior*, 58(2), 215-221.

Bingham, B., McFadden, K., Zhang, X., Bhatnagar, S., Beck, S., & Valentino, R. (2011) Early adolescence as a critical window during which social stress distinctly alters behavior and brain norepinephrine activity. *Neuropsychopharmacology*, 36, 896–909.

Bizot, J., Le Bihan, C., Puech, A., Hamon, M., & Thiébot, M. (1999) Serotonin and tolerance to delay of reward in rats. *Psychopharmacology*, 146, 400–412.

Bjorkqvist, K (2001) Social defeat as a stressor in humans. *Physiology & Behavior*. 73(3), 435–442

Blanchard, D., & Blanchard, R. (1988) Ethoexperimental approaches to the biology of emotion. *Annual Review of Psychology*, 39, 43–68.

Blanchard, D., Spencer, R., Weiss, S., Blanchard, R., Mcewen, B., & Sakai, R. (1995) Visible burrow system as a model of chronic social stress: Behavioral and neuroendocrine correlates. *Psychoneuroendocrinology*, 20(2), 117-134.

Blanchard, R., & Blanchard, D. (1977) Aggressive behavior in the rat. *Behavioral Biology*, 1, 197–224.

Blanchard, R., Wall, P. M., & Blanchard, D. (2003) Problems in the study of rodent aggression. *Hormones and Behavior*, 44, 161–170.

Bøen, E., Hummelen, B., Elvsåshagen, T., Boye, B., Andersson, S., Karterud, S., & Malt, U. (2015) Different impulsivity profiles in borderline personality disorder and bipolar II disorder. *Journal of Affective Disorders*, 170, 104-111.

Bolles, R., & Woods, P. (1964) The ontogeny of behaviour in the albino rat. *Animal Behaviour*, 12, 427-441.

Bölting, S., & von Engelhardt, N. (2017) Effects of the social environment during adolescence on the development of social behaviour, hormones and morphology in male zebra finches (*Taeniopygia guttata*). *Frontiers in Zoology*, 14:5.

Bourke, C., & Neigh, G. (2011) Behavioral effects of chronic adolescent stress are sustained and sexually dimorphic. *Hormones and Behavior*, 60(1),112-120.

Boutros, N., Der-Avakian, A., Semenova, S., Lee, S., & Markou, A. (2016) Risky choice and brain CRF after adolescent ethanol vapor exposure and social stress in adulthood. *Behavioural Brain Research*, 311, 160-166.

Bowman, R., Ferguson, D., & Luine, V. (2002) Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. *Neuroscience*, 113(2), 401-410.

Bowman, R., Zrull, M., & Luine, V. (2001) Chronic restraint stress enhances radial arm maze performance in female rats. *Brain Research*, 904, 279–289.

Brain, P. F. (1979) Hormones, drugs and aggression. *Annual research review*, 3, 1-38.

Brevers, D., Cleeremans, A., Verbruggen, F., Bechara, A., Kornreich, C., Verbanck, P., & Noël, X. (2012) impulsive action but not impulsive choice determines problem gambling severity. *PLOS ONE*, 7(11), e50647.

Brevet-Aebya, C., Brunelin, J., Iceta, S., Padovan, C., & Poulet, E. (2016) Prefrontal cortex and impulsivity: Interest of noninvasive brain stimulation. *Neuroscience & Biobehavioral Reviews*, 71, 112–134.

Bronson, F. H. (1973) Establishment of social rank among grouped male mice: Relative effects on circulating FSH, LH, and corticosterone. *Physiology & Behavior*, 10(5), 947-951.

Brown, I., & White, P. (1979) Serum electrolytes, lipids and cortisol in the African elephant, *Loxodonta Africana*. *Comparative Biochemistry and Physiology Part A: Physiology*. 62(4), 899-901.

Brunner, D., & Hen, R. (1997) Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. *Ann. N.Y. Acad. Sci*., 836, 81-105.

Burke, A., & Miczek, K. (2015) Escalation of cocaine self-administration in adulthood after social defeat of adolescent rats: Role of social experience and adaptive coping behavior. *Psychopharmacology*, 232(16), 3067–3079.

Butte, J., Kakihana, R., Farnham, M., & Noble, E. (1973) The relationship between brain and plasma corticosterone stress response in developing rats. *Endocrinology*, 92(6), 1775-1779.

Butts, K., Weinberg, J., Young, A., & Phillips, A. (2011) Glucocorticoid receptors in the prefrontal cortex regulate stress-evoked dopamine efflux and aspects of executive function. *PNAS*, 108(45), 18459-18464.

Caceres, S., Silvan, G., Martinez-Fernandez, L., Illera, M., Millan, P., Monsalve, B., Peña, L., & Illera, J. (2014) The effects of isoflavones on androgens and glucocorticoids during puberty on male Wistar rats. *Reproduction in Domestic Animals*, 49, 611–617.

Cardinal, R., Pennicott, D., Sugathapala, C., Robbins, T., & Everitt, B. (2001) Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science*, 292, 2499–2501.

Carlisle, N., & Rofes, E. (2007) School bullying: Do adult survivors perceive long-term effects? *Traumatology*, 13(1), 16-26.

Cavigelli, S. (1999) Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur Catta*. *Animal Behaviour*, 57(4), 935-944.

Cervantes, C., & Delville, Y. (2007) Individual differences in offensive aggression in golden hamsters: A model of reactive and impulsive aggression? *Neuroscience*, 150(3), 511–521.

Cervantes, C., Biggs E., & Delville, Y (2010) Differential responses to serotonin receptor ligands in an impulsive-aggressive phenotype. *Behavioral Neuroscience*, 124(4), 455– 469.

Cervantes, M., & Delville, Y. (2009) Serotonin 5-HT1A and 5-HT3 receptors in an impulsive-aggressive phenotype. *Behavioral Neuroscience*, 123(3), 589-598.

Chang, S. & Liang, K. (2017) The hippocampus integrates context and shock into a configural memory in contextual fear conditioning. *Hippocampus*, 27(2), 145-155

Chen, L., Shen, B., Liu, D., & Li, S. (2014) The effects of early-life predator stress on anxiety- and depression-like behaviors of adult rats. *Neural Plasticity*, 2014, 163908.

Cheung, T., & Cardinal, R. (2005) Hippocampal lesions facilitate instrumental learning with delayed reinforcement but induce impulsive choice in rats. *BMC Neuroscience*, 6, 36.

Chrousos GP (1995) The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *New England Journal of Medicine*, 332(20), 1351-62.

Chung, S., Son, H., & Kim, K. (2011) Circadian rhythm of adrenal glucocorticoid: Its regulation and clinical implications. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1812(5), 581–591.

Close, D., Yun, S., McCormick, S., Wildbill, A., & Li, W. (2010) 11-Deoxycortisol is a corticosteroid hormone in the lamprey. *Proceedings of the National Academy of Sciences*, 107(31), 13942–13947.

Coccaro, E. (1989) Central serotonin and impulsive aggression. *British Journal of Psychiatry*, 155(8), 52-62.

Cockrem, J., Barrett, D., Candy, E., & Potter, M. (2009) Corticosterone responses in birds: Individual variation and repeatability in Adelie penguins (*Pygoscelis adeliae*) and other species, and the use of power analysis to determine sample sizes. *General and Comparative Endocrinology*, 163, 158–168.

Cohn, L., Macfarlane, S., Yanez, C., & Imai, W. (1995) Risk-perception: Differences between adolescents and adults. *Health Psychology*, 14(3), 217-222.

Cole, B. & Robbins, T. (1989) Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats" implications for theories of selective attention and arousal. *Behavioural Brain Research*, 33(2), 165-179.

Collin, P., Tsang, W., & Metzger, J. (1984) Influence of stress on adrenocortical function in the male tree shrew (*Tupaia belangeri*) *General and Comparative Endocrinology*, 55(3), 450-457.

Conley, A. J., Plant, T. M., Abbott, D. H., Moeller, B. C., & Stanley, S. D. (2011) Adrenal androgen concentrations increase during infancy in male rhesus macaques (*Macaca mulatta*). *American journal of physiology. Endocrinology and metabolism*, 301(6), E1229-35.

Cooper, S., Goings, S., Kim, J., & Wood, R. (2014) Testosterone enhances risk tolerance without altering motor impulsivity in male rats. *Psychoneuroendocrinology*, 40, 201–212.

Coppens, C., de Boer, S., Buwalda, B., & Koolhaas, J. (2014) Aggression and aspects of impulsivity in wild-type rats. *Aggressive behavior*, 40(4), 300–308.

Coppens, C., de Boer, S., Steimer, T., & Koolhaas, J. M. (2012) Impulsivity and aggressive behavior in Roman high and low avoidance rats: Baseline differences and adolescent social stress induced changes. *Physiology and Behavior*, 105(5), 1156-1160.

Coppens, C., de Boer, S., Steimer, T., & Koolhaas, J. M. (2013) Correlated behavioral traits in rats of the Roman selection lines. *Behavioral Genetics*, 43, 220-226.

Costa, A., Benedetto, C., Fabris, C., Giraudi, G., Testori, O., Bertino, E., Marozio, L., Varvello, G., Arisio, R., Ariano, M., & Emanuel, A. (1996) Cortisol in human tissues at different stages of life. *Journal of Endocrinological Investigation*, 19(7), 463–471.

Cousins, M., Atherton, A., Turner, L., & Salamone, J. (1996) Nucleus accumbens dopamine depletions alter relative response allocation in a T-maze cost/benefit task. *Behavioural Brain Research*, 74, 189 197.

Covington III, H, & Miczek, K. (2001) Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". *Psychopharmacology*, 158, 388–398.

Covington III, H, & Miczek, K. (2005) Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: Dissociation from corticosterone activation. *Psychopharmacology (Berl)*, 183(3), 331-340.

Covington III, H., & Tropea, T. (2008) NMDA receptors in the rat VTA: A critical site for social stress to intensify cocaine taking. *Psychopharmacology (Berl)*, 197(2), 203–216.

Cragg, S. J., Baufreton, J., Xue, Y., Bolam, J. P. & Bevan, M. D. (2004) Synaptic release of dopamine in the subthalamic nucleus. *European Journal of Neuroscience*, 20, 1788-1802.

Cruz, F., DeLucia, R., & Planeta, C. (2005) Differential behavioral and neuroendocrine effects of repeated nicotine in adolescent and adult rats. *Pharmacology, Biochemistry and Behavior*, 80, 411– 417.

Cruz, F., Marin, M., Leão, R., & Planeta, C. (2012) Behavioral and neuroendocrine effects of the exposure to chronic restraint or variable stress in early adolescent rats. *International Journal of Developmental Neuroscience*, 30(1), 19-23.

Cruz, F., Quadros, I., Hogenelst, K., Planeta, C., & Miczek, K. (2011) Social defeat stress in rats: Escalation of cocaine and "speedball" binge self-administration, but not heroin. *Psychopharmacology*, 215(1), 165–175.

Curzon, P., Rustay, N., & Browman, K. (2009) Cued and contextual fear conditioning for rodents. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2009. Chapter 2. Available from: https://www.ncbi.nlm.nih.gov/books/NBK5223/

Czachowski, C., & Samson, H. (1999) Breakpoint determination and ethanol self-administration using an across- session progressive ratio procedure in the rat. *Alcoholism: clinical and experimental research*, 23(10), 1580-1586.

Dalle, M., & Delost, P. (1976) Plasma and adrenal cortisol concentrations in foetal, newborn and mother guinea-pigs during the perinatal period. *Journal of Endocrinology*, 70, 207-214.

Dalley, J. & Roiser, J. (2012) Dopamine, serotonin and impulsivity. *Neuroscience*, 215, 42-58.

Dalley, J., Fryer, T., Brichard, L., Robinson, E., Theobald, D., Lääne, K., Peña, Y., Murphy, E., Shah, Y., Probst, K., Abakumova, I., Aigbirhio, F., Richards, H., Hong, Y., Baron, J., Everitt, B., & Robbins, T. (2007) Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *SCIENCE*, 02, 1267-1270.

Dalley, J., Mar, A., Economidou, D., & Robbins, T. (2008) Neurobehavioral mechanisms of impulsivity: Fronto-striatal systems and functional neurochemistry. *Pharmacology Biochemistry & Behavior*, 90(2), 250-60.

Dalley, J.W., Everitt, B.J., & Robbins, T.W. (2011) Impulsivity, compulsivity, and top-down cognitive control. *Neuron.*, 69(4), 680-694.

Dambacher, F., Sack, A., Lobbestael, J., Arntz, A., Brugman, S., & Schuhmann, T. (2010) A network approach to response inhibition: Dissociating functional connectivity of neural components involved inaction restraint and action cancellation. *European Journal of Neuroscience*, 39, 821–831.

David, J., Cervantes, M., Trosky, K., Salinas, J., & Delville, Y. (2004) A neural network underlying individual differences in emotion and aggression in male golden hamsters. *Neuroscience*, 126, 567–578.

Davis, G., Stewart, A., Stanwood, G., Gowrishankar, R., Hahn, M., & Blakely, R. (2018) Functional coding variation in the presynaptic dopamine transporter associated with neuropsychiatric disorders drives enhanced motivation and context-dependent impulsivity in mice. *Behavioural Brain Research*, 337(30), 61-69.

Davis, M. (1992) The role of the amygdala in fear and anxiety. *Annu. Rev. Neurosci*., 15, 353-375.

De Bellis, M. (2002) Developmental traumatology: A contributory mechanism for alcohol and substance use disorders. *Psychoneuroendocrinology* 27, 155–170.

de Boer, S., Van Der Gugten, J., & Slangen, J. (1989) Plasma catecholamine and corticosterone responses to predictable and unpredictable noise stress in rats. *Physiology & Behavior*, 45, 789-795.

De Venter, M., Demyttenaere, K., & Bruffaerts, R. (2013) [The relationship between adverse childhood experiences and mental health in adulthood. A systematic literature review]. [Article in Dutch]. *Tijdschr Psychiatr*.,55(4), 259-68.

de Wit, H., Enggasser, J., & Richards, J. (2002) Acute administration of d-Amphetamine decreases impulsivity in healthy volunteers. *Neuropsychopharmacology*, 27, 813–825.

Delville, Y., De Vries, G., & Ferris, C. (2000) Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. *Brain, Behavior and Evolution*, 55(2), 53-76.

Delville, Y., Mansour, K. M., & Ferris, C. F. (1996). Serotonin blocks vasopressin-facilitated offensive aggression: Interactions within the ventrolateral hypothalamus of golden hamsters. *Physiology & Behavior*, 59, 813– 816.

Delville, Y., Melloni, R., & Ferris, C. (1998) Behavioral and neurobiological consequences of social subjugation during puberty in golden hamsters. *Journal of Neuroscience*, 18, 2667–2672.

Delville, Y., Stires, C., & Ferris, C. (1992) Distribution of corticotropin–releasing immunoreactivity in golden hamster brain. *Brain Research Bulletin*, 29, 681–684.

Der-Avakian, A., & Markou, A. (2011) The neurobiology of anhedonia and other reward-related deficits. *Trends in neurosciences*, 35(1), 68-77.

DiMeo, A., & Wood, R. (2004) Circulating androgens enhance sensitivity to testosterone self-administration in male hamsters. *Pharmacology Biochemistry and Behavior*, 79(2), 383–389.

Diorio, D., Viau, V., & Meaney, M. (1993) The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *Journal of Neuroscience*, 13(9), 3839-3847.

Dougherty, D., Bjork, J., Huckabee, H., Moeller, F., & Swann, A. (1999) Laboratory measures of aggression and impulsivity in women with borderline personality disorder. *Psychiatry Research*, 85, 315-326.

Dücker, G., Geyer, A., Schulze, I., & Stascheit, M. (1977) [Classical conditioning of agonistic behavior in the Syrian golden hamster (*Mesocricetus auratus* Waterhouse)]. *Zeitschrift für Tierpsychologie*, 45(4), 359-372.

Eagle, D. M., & Baunez, C. (2010) Is there an inhibitory-response-control system in the rat? Evidence from anatomical and pharmacological studies of behavioral inhibition. *Neurosci. Biobehav. Rev*., 34, 50–72.

Eagle, D., & Robbins, T. (2003) Lesions of the medial prefrontal cortex or nucleus accumbens core do not impair inhibitory control in rats performing a stop-signal reaction time task. *Behavioural Brain Research*, 146(1–2), 131-144.

Eagle, D., Bari, A., & Robbins, T. (2008) The neuropsychopharmacology of action inhibition: Cross-species translation of the stop-signal and go/no-go tasks. *Psychopharmacology*, 199, 439–456.

Eagle, D., Lehmann, O., Theobald, D., Pena, Y., Zakaria, R., Ghosh, R., Dalley, J., & Robbins, T. (2009) Serotonin depletion impairs waiting but not stop-signal reaction time in rats: Implications for theories of the role of 5-HT in behavioral inhibition. *Neuropsychopharmacology*, 34, 1311–1321

Eagle, D., Wong, J., Allan, M., Mar, A., Theobald, D., & Robbins, T (2011) Contrasting roles for dopamine D1 and D2 receptor subtypes in the dorsomedial striatum but not the nucleus accumbens core during behavioral inhibition in the stop-signal task in rats. *Journal of Neuroscience*, 31(20), 7349-7356.

Etain, B., Henry, C., Bellivier, F., Mathieu, F., & Leboyer, M. (2008) Beyond genetics: Childhood affective trauma in bipolar disorder. *Bipolar Disorders*, 10, 867–876.

Fadalti, M., Petraglia, F., Luisi, S., Bernardi, F., Casarosa, E., Ferrari, E., Luisi, M., Saggese, G., Genazzani, A., & Bernasconi, S. (1999) Changes of serum allopregnanolone levels in the first 2 years of life and during pubertal development. *Pediatric Research*, 46, 323–327.

Fadok, J., Dickerson, J., & Palmiter, R. (2009) Dopamine is necessary for cue-dependent fear conditioning. *Journal of Neuroscience*, 29(36), 11089-11097.

Fahlke, C., Engel, J., Eriksson, C., Hård, E., & Söderpalm, B. (1994) Involvement of corticosterone in the modulation of ethanol consumption in the rat. *Alcohol*, 11(3), 195-202.

Fahlke, C., Hård, E., Hansen, S. (1996) Facilitation of ethanol consumption by intracerebroventricular infusions of corticosterone. *Psychopharmacology*, 127, 133-139.

Feja, M., Hayn, L., & Koch, M. (2014) Nucleus accumbens core and shell inactivation differentially affects impulsive behaviours in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 54, 31–42.

Fendt, M & Fanselow, M.S. (1999) The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews*, 23, 743–760.

Feng, X., Wu, X., Morrill, R. J., Li, Z., Li, C., Yang, S., Li, Z., Cui, D., Lv, L., Hu, Z., Zhang, B., Yin, Y., Guo, L., Qin, D., & Hu, X. (2016) Social correlates of the dominance rank and long-term cortisol levels in adolescent and adult male rhesus macaques (*Macaca mulatta). Scientific reports*, 6, 25431.

Feola, T., de Wit, H., & Richards, J. (2000) Effects of d-Amphetamine and alcohol on a measure of behavioral inhibition in rats. *Behav Neurosc*i, 114, 838–848.

Ferris, C., Gold, L., De Vries, G. J., & Potegal, M. (1990), Evidence for a functional and anatomical relationship between the lateral septum and the hypothalamus in the control of flank marking behavior in golden hamsters. *J. Comp. Neurol*., 293, 476-485.

Ferris, C. F., Melloni, R. H., Jr., Koppel, G., Perry, K. W., Fuller, R. W., & Delville, Y. (1997). Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. *Journal of Neuroscience*, 17, 4331– 4340.

Ferris, C., Messenger, T., & Sullivan, R. (2005) Behavioral and neuroendocrine consequences of social subjugation across adolescence and adulthood. *Frontiers in Zoology*, 5, 2-7.

Fleck, D., Kotwal, R., Eliassen, J., Lamy, M., Delbello, M., Adler, C., Durling, M., Cerullo, M., & Strakowski, S. (2011) Preliminary evidence for increased frontosubcortical activation on a motor impulsivity task in mixed episode bipolar disorder. *Journal of Affective Disorders*, 133(1-2), 333–339.

Fletcher, P., Tampakeras, M., Sinyard, J., & Higgins, G. (2007) Opposing effects of 5-HT2A and 5-HT2C receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. *Psychopharmacology*, 195(2), 223–234.

Fodor, A., Barsvari, B., Aliczki, M., Balogh, Z., Zelena, D., Goldberg, S. R. & Haller, J. (2014) The effects of vasopressin deficiency on aggression and impulsiveness in male and female rats. *Psychoneuroendocrinology*, 47, 141-150.

Fonseca, M., Murakami, M., & Mainen, Z. (2015) Activation of dorsal raphe serotonergic neurons promotes waiting but is not reinforcing. *Current Biology*, 25(3), 306-315.

Frischknecht, H., Siegfried, B., & Waser, P. (1982) Learning of submissive behavior in mice: A new model. *Behavioural Processes*, 7, 235-245.

Fujino, K., Yoshitake, T., Inoue, O., Ibii, N., Kehr, J., Ishida, J., Nohta, H., & Yamaguchi, M. (2002) Increased serotonin release in mice frontal cortex and hippocampus induced by acute physiological stressors. *Neurosci. Lett.*, 320, 91-95.

Gerlach, J., & McEwen, B. (1972) Rat brain binds adrenal steroid hormone: Radioautography of hippocampus with corticosterone. *Science*, 175(4026), 1133-1136.

Giedd, J., Blumenthal, J., Jeffries, N., Castellanos, F., Liu, H., Zijdenbos, A., Paus, T., Evans, A., & Rapoport, J. (1999) Brain development during childhood and adolescence: A longitudinal MRI study. *Nature Nuroscience*, 2(10), 861-863.

Gladstone, G., Parker, G. & Malhi, G. (2006) Do bullied children become anxious and depressed adults? A cross-sectional investigation of the correlates of bullying and anxious depression. *The Journal of Nervous and Mental Disease*, 194 (3), 201-208.

Goldman, L., & Swanson, H. (1975) Developmental changes in pre-adult behavior in confined colonies of golden hamsters. *Developmental psychobiology*, 8(2), 137–150.

Goldman, L., Winget, C., Hollingshead, G., & Levine, S. (1973) Postweaning development of negative feedback in the Pituitary-Adrenal system of the rat. *Neuroendocrinology*, 12, 199-211.

Gomez, D.M., & Newman, S. (1992) Differential projections of the anterior and posterior regions of the medial amygdaloid nucleus in the Syrian hamster. J. *Comp. Neurol*., 317: 195-218.

Grant, E., & Chance, M. (1958) Rank order in caged rats. *Animal Behaviour*, 6, 183-194.

Green, L., & Myerson, J. (2004) A discounting framework for choice with delayed and probabilistic rewards. *Psychological bulletin*, 130(5), 769-92.

Green, L., Fry, A. & Myerson, J. (1994) Discounting of delayed rewards: A life-span comparison. *Psychological Science*, 5(1), 33-36.

Guillet, R., Saffran, M., & Michaelson, S. (1980) Pituitary-adrenal response in neonatal rats. *Endocrinology*, 106, 991–994.

Guillette, L., Woodward, A., Crain, D., Masson, G., Palmer, B., Cox, M., You-Xiang, Q., & Orlando, E. (1997) The reproductive cycle of the female american alligator (*Alligator mississippiensis*) *General and Comparative Endocrinology*, 108, 87–101.

Gunnar, M.R., Wewerka, S., Frenn, K., Long, J.D., & Griggs, C. (2009) Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: Normative changes and associations with puberty. *Dev Psychopathol*., 21(1), 69-85.

Gustafson, A., & Belt, W., (1981) The adrenal cortex during activity and hibernation in the male little brown bat, *Myotis lucifugus lucifugus*: Annual rhythm of plasma cortisol levels. *General and Comparative Endocrinology*, 44, 269–278.

Hadamitzky, M., & Koch, M. (2009) Effects of acute intra-cerebral administration of the 5-HT2A/C receptor ligands DOI and ketanserin on impulse control in rats. *Behavioural Brain Research*, 204, 88–92.

Haller, J., & Bakos, N. (2002) Stress-induced social avoidance: A new model of stress-induced anxiety? *Physiology & Behavior*, 77 (2–3), 327-332.

Haller, J., Harold, G., Sandi, C., & Neumann, I. D. (2014) Effects of adverse early-life events on aggression and anti-social behaviours in animals and humans. *Journal of Neuroendocrinology*, 26, 724–738.

Haller, J., van de Schraaf, J., & Kruk, M. (2001) Deviant forms of aggression in glucocorticoid hyporeactive rats: A model for `pathological' aggression? *Journal of Neuroendocrinology*, 13, 102-107.

Harrison, A., Everitt, B., & Robbins, T. (1997a) Doubly dissociable effects of median- and dorsal-raphé lesions on the performance of the five-choice serial reaction time test of attention in rats. *Behavioural Brain Research*, 89 (1–2),135-149.

Harrison, A., Everitt, B., & Robbins, T. (1997b) Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: Interactions with dopaminergic mechanisms. *Psychopharmacology*, 33, 329–342.

Harrison, A., Everitt, B., & Robbins, T. (1999) Central serotonin depletion impairs both the acquisition and performance of a symmetrically reinforced Go/No-Go conditional visual discrimination. *Behavioural Brain Research*, 100, 99–112.

Hayton, S., Maracle, A., & Olmstead, M (2012) Opposite effects of amphetamine on impulsive action with fixed and variable delays to respond. *Neuropsychopharmacology*, 37, 651–659.

Hecht, L., & Latzman, R. (2015) Revealing the nuanced associations between facets of trait impulsivity and reactive and proactive aggression. *Personality and Individual Differences*, 83, 192–197.

Hench, P. Kendall, E., Slocumb, C., & Polley, H. (1949) The effect of a hormone of the adrenal cortex (17-hydroxy-ll-dehydrocorticosterone: Compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis. *Annals of the rheumatic diseases*, 8, 97-104.

Henning, S., & Genovese, G. (1985) Postnatal development of cholesterol ester hydrolase activity in the rat adrenal. *J. Steroid Biochem.*, 22(6), 803-808.

Henning, S.J. (1978) Plasma concentrations of total and free corticosterone during development in the rat. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 235(5):E451-6.

Herman, J., & Perry, C. (1989) Childhood trauma in borderline personality disorder. *American Journal of Psychiatry*, 146(4), 490-495.

Hershenberg, R., Satterthwaite, T., Daldal, A., Katchmar, N., Moore, T., Kable, J., & Wolf, D. (2016) Diminished effort on a progressive ratio task in both unipolar and bipolar depression. *Journal of Affective Disorders*, 196, 97–100.

Hodges, T., & McCormick, C. (2015) Adolescent and adult male rats habituate to repeated isolation, but only adolescents sensitize to partner unfamiliarity. *Hormones and Behavior*, 69, 16–30.

Hodos, W. (1961) Progressive ratio as a measure of reward strength. *Science*, 134 (3483), 943-944.

Holson, R., & Pearce, B. (1992) Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol. Teratol*., 14, 221-228.

Hovens, J., Wiersma, J., Giltay, E., van Oppen, P., Spinhoven, P., Penninx, B., & Zitman, F. (2010) Childhood life events and childhood trauma in adult patients with depressive, anxiety and comorbid disorders vs. controls. *Acta Psychiatrica Scandinavica,* 122, 66–74.

Huhman, K., Moore, T. Ferris, C., Mougey, E., & Meyerhoff, J. (1991) Acute and repeated exposure to social conflict in male golden hamsters: Increases in plasma POMC-peptides and cortisol and decreases in plasma testosterone. *Hormones and Behavior*, 25(2), 206-216.

Idler, D., Sangalang, G., & Truscott, B. (1972) Corticosteroids in the South American Lungfish. *General and comparative endocrinology supplement*, 3, 238-244.

Ilin, Y., & Richter-Levin, G. (2009) Enriched environment experience overcomes learning deficits and depressive-like behavior induced by juvenile stress. *PLOS ONE*, 4(1): e4329.

Iñiguez, S, Riggs, L., Nieto, S., Dayrit, G., Zamora, N., Shawhan, K., Cruz, B., & Warren, B. (2014) Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. *Stress*, 17(3), 247-55.

Isgor, C., Kabbaj, M., Akil, H., & Watson, S.J. (2004) Delayed effects of chronic variable stress during peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. *Hippocampus*, 14, 636–648.

Izquierdo, A., & Jentsch, J. D. (2012) Reversal learning as a measure of impulsive and compulsive behavior in addictions. *Psychopharmacology*, 219(2), 607-620.

Jack, K., Schoof, V., Sheller, C., Rich, C., Klingelhofer, P., Ziegler, T., & Fedigan, L. (2014) Hormonal correlates of male life history stages in wild white-faced capuchin monkeys (*Cebus capucinus*). *General and Comparative Endocrinology*, 195, 58-67.

Jackson, R., Alexander, J., & Maier, S. (1980) Learned helplessness, inactivity, and associative deficits: Effects of inescapable shock on response choice escape learning. *Journal of Experimental Psychology: Animal Behavior Processes*, 6(1), 1-20.

Jacob, G., Gutz, L., Bader, K., Lieb, K., Tüscher, O., & Stahl, C. (2010) Impulsivity in borderline personality disorder: Impairment in self-report measures, but not behavioral inhibition. *Psychopathology*, 43, 180-188.

Jacobson-Pick, S., & Richter-Levin, G. (2010) Differential impact of juvenile stress and corticosterone in juvenility and in adulthood, in male and female rats. *Behavioural Brain Research*, 214, 268–276.

Jasnow, A., Banks, M., Owens, E., & Huhman, K. (1999) Differential effects of two corticotropin-releasing factor antagonists on conditioned defeat in male Syrian hamsters (*Mesocricetus auratus*) *Brain Research*, 846(1), 122–128.

Jentsch, D., Ashenhurst, J. R., Cervantes, M. C., Groman, S. M., James, A., S., & Pennington, Z. T., (2014) Dissecting impulsivity and its relationships to drug addictions. *Ann N.Y Acad Sci*, 1327, 1–26.

Joels, M., & de Kloet, R. (1994) Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. *Progress in Neurobiology*, 43, 1-36.

Jones, M., Hillhouse, E., & Burden, J. (1977) Dynamics and mechanics of corticosteroid feedback at the hypothalamus and anterior pituitary gland. *Journal of Endocrinology*, 73, 405-417. Google Scholar Abstract.

Jupp, B., Caprioli, D., Saigal, N., Reverte, I., Shrestha, S., Cumming, P., Everitt, B., Robbins, T. & Dalley, J. (2013), Dopaminergic and GABA-ergic markers of impulsivity in rats: Evidence for anatomical localization in ventral striatum and prefrontal cortex. *Eur J Neurosci*, 37, 1519-1528.

Kattesh H., Charles, S., Baumbach, G., & Gillespie, B. (1990) Plasma cortisol distribution in the pig from birth to six weeks of age. *Neonatology*, 58, 220-226.

Kestenberg, J. (1968) Phases of adolescence: With suggestions for a correlation of psychic and hormonal organizations: Part III puberty growth, differentiation, and consolidation. *Journal of the American Academy of Child Psychiatry*, 7(1), 108-151.

Kheramin, S., Body, S., Ho, M., Velázquez-Martinez, D., Bradshaw, C., Szabadi, E., Deakin, J., & Anderson, I. (2004) Effects of orbital prefrontal cortex dopamine depletion on inter-temporal choice: A quantitative analysis. *Psychopharmacology*, 175, 206–214.

Kiess, W., Meidert, A., Dressendorfer, R., Schriever, K., Kessler, U., Konig, A., Schwarz, H., & Strasburger, C. (1995) Salivary cortisol levels throughout childhood and adolescence: Relation with age, pubertal stage, and weight. *Pediatric Research*, 37(4), 502-506.

Kim, J. J., & Jung, M. W. (2005) Neural circuits and mechanisms involved in Pavlovian fear conditioning: A critical review. *Neuroscience and biobehavioral reviews*, 30(2), 188-202.

Knutsson, U., Dahlgren, J., Marcus, C., Rosberg, S., Brönnegård, M., Stierna, P., & Albertsson-Wikland, K. (1997) Circadian cortisol rhythms in healthy boys and girls: Relationship with age, growth, body composition, and pubertal development. *The Journal of Clinical Endocrinology & Metabolism*, 82(2), 536–540.

Kockler, T., Stanford, M., Nelson, C., Meloy, J., & Sanford, K. (2006) Characterizing aggressive behavior in a forensic population. *American Journal of Orthopsychiatry*, 76(1), 80 – 85.

Koolhaas, J., Meerlo, P., De Boer, S., Strubbe, J., & Bohus, B. (1997) The temporal dynamics of the stress response. *Neuroscience and Biobehavioral Reviews*, 21(6), 775–782.

Koskinen, T., & Sirviö, J. (2001) Studies on the involvement of the dopaminergic system in the 5-HT2 agonist (DOI)-induced premature responding in a five-choice serial reaction time task. *Brain Research Bulletin*, 54 (1), 65–75.

Koskinen, T., Ruotsalainen, S., & Sirviö, J. (2000) The 5-HT2 receptor activation enhances impulsive responding without increasing motor activity in rats. *Pharmacology Biochemistry and Behavior*, 66(4), 729-738.

Kovalenko, I., Galyamina, A.G., Smagin, D., Michurina, T., Kudryavtseva, N., & Enikolopov, G. (2014) Extended effect of chronic social defeat stress in childhood on behaviors in adulthood. *PLOS ONE*, 9 (3), e91762.

Kramer, T., & Rilling, M. (1970) Differential reinforcement of low rates: A selective critique. *Psychological Bulletin*, 74(4), 225-2554.

Kretz, O., Reichardt, H., Schutz, G., & Bock, R. (1999) Corticotropin-releasing hormone expression is the major target for glucocorticoid feedback-control at the hypothalamic level. *Brain Research*, 818(2), 488-491.

Lachuer, J., Delton, I., Buda, M., & Tappaz, M. (1994) The habituation of brainstem catecholaminergic groups to chronic daily restraint stress is stress specific like that of the hypothalamo-pituitary-adrenal axis. *Brain Research*, 638, 196-202.

Lance VA, Lauren D. (1984) Circadian variation in plasma corticosterone in the American alligator, *Alligator mississippiensis*, and the effects of ACTH injections. *General and Comparative Endocrinology*, 54(1), 1-7.

Larson, R., & Richards, M. (1991) Daily companionship in late childhood and early adolescence: Changing developmental contexts. *Child Development*, 62(2), 284–300.

Látalová, K. (2009) Bipolar disorder and aggression. *Int J Clin Pract* , 63(6), 889–899.

Laudenslager, M. L., Jorgensen, M. J., & Fairbanks, L. A. (2012) Developmental patterns of hair cortisol in male and female nonhuman primates: Lower hair cortisol levels in vervet males emerge at puberty. *Psychoneuroendocrinology*, 37(10), 1736-1739.

Lawrence, K., Allen, J., & Chanen, A. (2010) Impulsivity in borderline personality disorder: Reward-based decision-making and its relationship to emotional distress. *Journal of Personality Disorders*, 24(6), 786–799.

Leboulenger, F., Delarue, C., Tonon, M., Jegou, S., Leroux, P., & Vaudry, H., (1979) Seasonal study of the interrenal function of the european green frog, in vivo and in vitro. *General and Comparative Endocrinology*, 39, 388–396.

Legro, R., Lin, H., Demers, L., & Lloyd, T. (2003) Urinary Free Cortisol Increases in Adolescent Caucasian Females during Perimenarche. *The Journal of Clinical Endocrinology & Metabolism*, 88(1), 215–219.

Lehmann, J., & Feldon, J. (2000) Long-term biobehavioral effects of maternal separation in the rat: Consistent or confusing? *Reviews in Neurosciences*, 11, 383-408.

Leisman, G., Melillo, R., & Carrick, F (2013) Clinical motor and cognitive neurobehavioral relationships in the basal ganglia, basal ganglia - an integrative view. Fernando A. Barrios and Clemens Bauer, IntechOpen, DOI:10.5772/55227. Available from: https://www.intechopen.com/books/basal-ganglia-an-integrative-view/clinical-motor-and-cognitive-neurobehavioral-relationships-in-the-basal-ganglia

Lerwill, C.J., & Makings, P. (1971) The agonistic behavior of the golden hamster *Mesocricetus auratus* (Waterhouse) *Animal Behaviour*, 19, 714– 721.

Licht, P., McCreery, B., Barnes, R., & Pang, R. (1983) Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana*. *General and Comparative Endocrinology*, 50 (1), 124–145.

Liu, Y., Wilkinson, L., & Robbins, T. (2004) Effects of acute and chronic buspirone on impulsive choice and efflux of 5-HT and dopamine in hippocampus, nucleus accumbens and prefrontal cortex. *Psychopharmacology*, 173, 175–185.

Logan, G., Schachar, R., & Tannock, R. (1997) Impulsivity and inhibitory control. *Psychological science*, 8(1), 60-64.

Lopez, M., Doremus-Fitzwater, T., & Becker, H. (2011) Chronic social isolation and chronic variable stress during early development induce later elevated ethanol intake in adult C57BL/6J mice. *Alcohol*, 45(4), 355–364.

Luo, M., Zhou, J., & Liu, Z. (2015) Reward processing by the dorsal raphe nucleus: 5-HT and beyond. *Learning & memory (Cold Spring Harbor, N.Y.)*, 22(9), 452-60.

Madden, G.J., Ewan, E.E., & Lagorio, C.H.J. (2007) Toward an animal model of gambling: delay discounting and the allure of unpredictable outcomes. *Journal of Gambling Studies*, 23(1), 63–83.

Magariños, A., Somoza, G., & De Nicola, A. (1987) Glucocorticoid negative feedback and glucocorticoid receptors after hippocampectomy in rats. *Horm Metab Res*., 19(3), 105-109.

Mahmoud, S., Scaccianoce, S., Scraggs, P., Nicholson, S., Gillham, B., & Jones, T. (1984) Characteristics of corticosteroid inhibition of adrenocorticotrophin release from the anterior pituitary gland of the rat. *Journal of Endocrinology*., 102, 33-42. Google Scholar Abstract.

Maren, Stephen (2001) Neurobiology of pavlovian fear conditioning. *Annual Review of Neuroscience*, 24, 897-93.

Mariano, T., Bannerman, D., McHugh, S., Preston, T., Rudebeck, P., Rudebeck, S., Rawlins, J., Walton, M., Rushworth, M., Baxter, M., & Campbell, T. (2009) Impulsive choice in hippocampal but not orbitofrontal cortex-lesioned rats on a nonspatial decision-making maze task. *European Journal of Neuroscience*, 30, 472–484.

Marin, M., Cruz, F., & Planeta, C. (2007) Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. *Physiology & Behavior*, 90(1), 29-35.

Márquez, C., Poirier, G.L., Cordero, M.I., Larsen, M.H., Groner, A., Marquis, J., Magistretti, P. J., Trono, D., & Sandi, C. (2013) Peripuberty stress leads to abnormal aggression, altered amygdala and orbitofrontal reactivity and increased prefrontal MAOA gene expression. *Translational Psychiatry*, 3, e216.

Martin N. Muller · Richard W. Wrangham (2004) Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). *Behavioral Ecology and Sociobiology*, 55, 332–340.

Martín‐Blanco, A., Ferrer, M., Soler, J., Arranz, M.J., Vega, D., Calvo, N., Elices, M., Sanchez‐Mora, C., García‐Martinez, I., Salazar, J., Carmona, C., Bauzà, J., Prat, M., Pérez, V., & Pascual, J.C. (2016) The role of hypothalamus–pituitary–adrenal genes and childhood trauma in borderline personality disorder. *European Archives of Psychiatry and Clinical Neuroscience*, 266, 307–316.

Masaki, D., Yokoyama, C., Kinoshita, S., Tsuchida, H., Nakatomi, Y., Yoshimoto, K., & Fukui, K. (2006) Relationship between limbic and cortical 5-HT neurotransmission and acquisition and reversal learning in a go/no-go task in rats. *Psychopharmacology*, 189(2), 249–258.

Mathews, I., Wilton, A., Styles, A., & McCormick, C. (2008) Increased depressive behaviour in females and heightened corticosterone release in males to swim stress after adolescent social stress in rats. *Behavioural Brain Research*, 190(1), 33–40.

Matsumoto, M., Togashi, H., Konno, K., Koseki, H., Hirata, R., Izumi, T., Yamaguchi, T., & Yoshioka, M. (2008) Early postnatal stress alters the extinction of context-

dependent conditioned fear in adult rats. *Pharmacology Biochemistry and Behavior*, 89(3), 247-252.

McCormick, C. (2010) An animal model of social instability stress in adolescence and risk for drugs of abuse. *Physiology & Behavior*, 99(2), 194-203.

McCormick, C., Thomas, C., Sheridan, C., Nixon, F., Flynn, J., & Mathews, I. (2012) Social instability stress in adolescent male rats alters hippocampal neurogenesis and produces deficits in spatial location memory in adulthood. *Hippocampus*, 22, 1300–1312.

McDevitt, R., Tiran-Cappello, A., Shen, H., Balderas, I., Britt, J., Marino, R., Chung, S., Richie, C., Harvey, B., & Bonci, A. (2014) Serotonergic versus nonserotonergic dorsal raphe projection neurons: Differential participation in reward circuitry. *Cell Reports*, 8(6), 1857-1869.

McEwen, B., Weiss, J., & Schwartz, L. (1969) Uptake of corticosterone by rat brain and its concentration by certain limbic structures. *Brain Research*, 16(1), 227–241

Mclaughlin, K., Baran, S., Wright, R., & Conrad, C. (2006) Chronic stress enhances spatial memory in ovariectomized female rats despite CA3 dendritic retraction: possible involvement of CA1 neurons. *Neuroscience*, 135(4), 1045-54.

Meaney, M., Sapolsky, R., & McEwen, B. (1985) The development of the glucocorticoid receptor system in the rat limbic brain. I. Ontogeny and autoregulation. *Brain Research*, 350(1-2), 159-64.

Meerlo, P., Over, G., Daana, S., Van den Hoofdakker, R., & Koolhaas, J. (1996) Changes in behaviour and body weight following a single or double social defeat in rats. *Stress*, 1, 21-32.

Mendle, J. (2014) Why puberty matters for psychopathology? *Child Development Perspectives*, 8(4), 218–222.

Midgley, S., Heather, N., & Davies, J. (2001) Levels of aggression among a group of anabolic-androgenic steroid users. *Medicine, Science and the Law*, 41(4), 309-14.

Mitchell, C. & Flaherty, C. (2005) Differential effects of removing the glucose or saccharin components of a glucose–saccharin mixture in a successive negative contrast paradigm. *Physiology & Behavior*, 84, 579 – 583

Mitra, R., Sundlass, K., Parker, K., Schatzberg, A., & Lyons, D. (2006) Social stress-related behavior affects hippocampal cell proliferation in mice. *Physiology & Behavior*, 89(2), 123-127.

Miyazaki, K. W., Miyazaki, K. & Doya, K. (2011a), Activation of the central serotonergic system in response to delayed but not omitted rewards. *European Journal of Neuroscience*, 33, 153-160.

Miyazaki, K. W., Miyazaki, K. & Doya, K. (2011b) Activation of dorsal raphe serotonin neurons underlies waiting for delayed rewards. *Journal of Neuroscience*, 31(2) 469-479.

Miyazaki, K., Miyazaki, K. W., Yamanaka, A., Tokuda, T., Tanaka, K. F., & Doya, K. (2018) Reward probability and timing uncertainty alter the effect of dorsal raphe serotonin neurons on patience. *Nature Communications*, 9, 1-11.

Miyazaki, K., Miyazaki, K., & Doya, K. (2012) The role of serotonin in the regulation of patience and impulsivity. *Molecular Neurobiology*, 45, 213–224.

Miyazaki, K., Miyazaki, W, & Matsumoto, G. (2004) Diferent representation of forthcoming reward in nucleus accumbens and medial prefrontal cortex. *Neuroreport*, 15, 721–726.

Mobini, S., Chiang, T., Al-Ruwaitea, A., Ho, M., Bradshaw, C., & Szabadi, E. (2000a) Effect of central 5-hydroxytryptamine depletion on inter-temporal choice: A quantitative analysis. *Psychopharmacology*, 149(3), 313–318.

Mobini, S., Chiang, T., Ho, M, Bradshaw, C., & Szabadi, E. (2000b) Effects of central 5-hydroxytryptamine depletion on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology*, 152(4), 390–397.

Mommsen, T., Vijayan, M., & Moon, T. (1999) Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries*, 9, 211–268.

Morein-Zamir, S., & Robbins, T. (2015) Fronto-striatal circuits in response-inhibition: Relevance to addiction. *Brain Research*, 1628, Part A, 117-129.

Moreno, M., Cardona, D., Gómez, M. J., Sánchez-Santed, F., Tobeña, A., Fernández-Teruel, A., Campa, L., Suñol, C., Escarabajal, M. D., Torres, C., & Flores, P. (2010) Impulsivity characterization in the Roman high- and low-avoidance rat traits: Behavioral and neurochemical differences. *Neuropsychopharmacology*, 35, 1198-1208.

Moyer, K. E. (1968) Kinds of aggression and their physiological basis. *Communications in behavioral biology, Part A*, 2, 65-87

Müller, M. S.; Porter, E. T., Grace, J. K., Awkerman, J. A., Birchler, K. T., Gunderson, A. R., Schneider, E. G., Westbrock, M. A., & Anderson, D. J. (2011) Maltreated nestlings exhibit correlated maltreatment as adults: Evidence of a "cycle of violence" in Nazca Boobies (Sula granti). *The Auk*, 128(4), 615-619.

Munck, A. (1971) Glucocorticoid inhibition of glucose uptake by peripheral tissues: Old and new evidence, molecular mechanisms, and physiological significance. *Perspectives in Biology and Medicine*, 14(2), 265-289.

Nakayasu, Y., & Ishii, K. (2008) Effects of pair-housing after social defeat experience on elevated plus-maze behavior in rats. *Behavioural Processes*, 78, 477–480.

Nansel, T. R., Overpeck, M., Pilla, R. S., Ruan, W. J., Simons-Morton, B., & Scheidt, P. (2001) Bullying behaviors among US youth: Prevalence and association with psychosocial adjustment. *JAMA*, 285(16), 2094-2100.

Nautiyal, K., Tanaka, K., Barr, M., Tritschler, L., Le Dantec, Y., David, D., Gardier, A., Blanco, C., Hen, R., & Ahmari, S. (2015) Distinct circuits underlie the effects of 5-ht1b receptors on aggression and impulsivity. *Neuron*, 86(3), 813-26.

Navarra, R., Comery, T., Graf, R., Rosenzweig-Lipson, S., & Day, M. (2008) The 5-HT2C receptor agonist WAY-163909 decreases impulsivity in the 5-choice serial reaction time test. *Behavioural Brain Research*, 188(2), 412-415.

Nelson, R.J. (2005) An *Introduction to Behavioral Endocrinology*. Third ed. Sunderland, MA, USA: Sinauer Associates.

Netherton, C, Goodyer, I., Tamplin, A., & Herbert, J. (2004) Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender. *Psychoneuroendocrinology*, 29, 125–140.

Newman, J., Widom, C., & Nathan, S. (1985) Passive avoidance in syndromes of disinhibition: Psychopathy and extraversion. *Journal of Personality and Social Psychology,* 48(5), 1316–1327.

Newman, M., Holden, G., & Delville, Y. (2005) Isolation and the stress of being bullied. *Journal of Adolescence*, 28, 343–357.

Noori, H., Helinski, S., & Spanagel, R. (2014) Cluster and meta-analyses on factors influencing stress-induced alcohol drinking and relapse in rodents. *Addiction Biology*, 19, 225–232.

Nussey S, Whitehead S. *Endocrinology: An Integrated Approach*. Oxford: BIOS Scientific Publishers; 2001. Chapter 4, The adrenal gland. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26/?report=classic

Nylander, I., & Roman, E. (2013) Is the rodent maternal separation model a valid and effective model for studies on the early-life impact on ethanol consumption? *Psychopharmacology (Berl*), 229(4), 555–569.

Oddie, C., Blaine, H., Bradshaw, D., Coghlan, P., Denton, A., Nelson, F., & Scoggins, A. (1976) Blood corticosteroids in australian marsupial and placental mammals and one monotreme. *Journal of Endocrinology*, 69, 341-348.

Ohmura, Y., Kumamoto, H., Tsutsui-Kimura, I., Minami, M., Izumi, T., Yoshida, T., & Yoshioka, M. (2013) Tandospirone suppresses impulsive action by possible blockade of the 5-HT1A receptor. *Journal of Pharmacological Sciences*, 122(2), 84-92.

Ordyan, N., Galeeva, A., & Pivina, S. (2008) Expression of glucocorticoid receptor in the brain of rats during postnatal ontogeny. *Bulletin of Experimental Biology and Medicine*, 146(2), 176-179.

Oskis, A., Loveday, C., Hucklebridge,F., Thorn, L., & Clow, A. (2009) Diurnal patterns of salivary cortisol across the adolescent period in healthy females. *Psychoneuroendocrinology*, 34 (3), 307-316.

Ottenweller, J., Tapp, W., Burke,J., & Natelson, B. (1985) Plasma cortisol and corticosterone concentrations in the golden hamster, (*Mesocricetus Auratus*) *Life Sciences*, 37(16), 1551-1558.

Øverli, Ø., Korzan, W., Höglund, E., Winberg, S., Bollig, H., Watt, M., Forster, G., Barton, B., Øverli, E., Renner, K., & Summers, C. (2004) Stress coping style predicts aggression and social dominance in rainbow trout. *Hormones and Behavior*, 45(4), 235-241.

Pacák, K., & Palkovits, M. (2001) Stressor specificity of central neuroendocrine responses: Implications for stress-related disorders. *Endocrine Reviews*, 22(4), 502–548.

Parker, A. J., & Newsome, W. T (1998) Sense and the single neuron: Probing the physiology of perception. *Annual Review of Neuroscience*, 21, 227–277.

Passetti, F., Dalley, J., & Robbins, T. (2003) Double dissociation of serotonergic and dopaminergic mechanisms on attentional performance using a rodent five-choice reaction time task. *Psychopharmacology*, 165(2), 136–145.

Pattij, T., & Vanderschuren, L.J.M.J. (2008) The neuropharmacology of impulsive behavior. *Trends Pharmacol Sci*, 29(4), 192-199.

Pattij, T., Broersen, L., van der Linde, J., Groenink, L., van der Gugten, J., Maes, R., & Olivier, B. (2003) Operant learning and differential-reinforcement-of-low-rate 36-s responding in 5-HT1A and 5-HT1B receptor knockout mice. *Behavioural Brain Research*, 141(2), 137-145.

Pattij, T., Janssen, M., Vanderschuren, L., Schoffelmeer, A., & van Gaalen, M. (2007) Involvement of dopamine D1 and D2 receptors in the nucleus accumbens core and shell in inhibitory response control. *Psychopharmacology*, 191(3), 587–598.

Pecoraro, N., & Dallman, M. (2005) c-Fos after incentive shifts: Expectancy, incredulity, and recovery. *Behavioral Neuroscience*, 119(2), 366 –387.

Pellis, S.M., & Pellis, V.C. (1988a) Play-fighting in the Syrian golden hamster *Mesocricetus auratus* Waterhouse, and its relationship to serious fighting during postweaning development. *Developmental Psychobiology*, 21, 323–337.

Pellis, S.M., & Pellis, V.C., (1988b) Identification of the possible origin of the body targets that differentiate play fighting from serious fighting in Syrian golden hamsters (*Mesocricetus auratus*) *Aggressive Behavior*, 14, 437–449.

Pezze, M., & Feldon, J. (2004) Mesolimbic dopaminergic pathways in fear conditioning. *Progress in Neurobiology*, 74(5), 301-320.

Pfaff, D., Arnold, A., Etgen, A., Fahrbach, S., & Rubin, R. (Ed.) (2002) *Hormones, Brain and Behavior*. USA: Academic Press

Phillips, R.G., & LeDoux, J.E. (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci*., 106(2), 274-85.

Pignatelli, D., Xiao, F., Gouveia, A., Ferreira, J., & Vinson, G. (2006) Adrenarche in the rat. *Journal of Endocrinology*, 191, 301–308.

Pitman, D., Ottenweller, J., & Benjamin, N. (1988) Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: Chronic stress and habituation. *Physiology & Behavior*, 43, 47-55.

Potegal, M., Huhman, K., Moore, T., & Meyerhoff, J. (1993) Conditioned defeat in the Syrian golden hamster (*Mesocricetus auratus*) *Behavioral and Neural Biology*, 60(2), 93-102.

Pynoos, R., Ritzmann, R., Steinberg, A., Goenjian, A., & Prisecaru, I. (1996) A behavioral animal model of posttraumatic stress disorder featuring repeated exposure to situational reminders. *Biological Psychiatry*, 39(2), 129-134.

Quevedo, K. M., Benning, S. D., Gunnar, M. R., & Dahl, R. E. (2009) The onset of puberty: effects on the psychophysiology of defensive and appetitive motivation. *Development and psychopathology*, 21(1), 27-45.

Rachlin, H., Raineri, A., & Cross, D. (1991) Subjective probability and delay. *Journal of the experimental analysis of behavior*, 55(2), 233-244.

Rasmussen, L., & Crow, G. (1993) Serum corticosterone concentrations in immature captive whitetip reef sharks, *Triaenodon obesus*. *Endocrinology,* 267(3), 283–287.

Razzoli, M., Carboni, L., Guidi, A., Gerrard, P., & Arban, R. (2007) Social defeat-induced contextual conditioning differentially imprints behavioral and adrenal reactivity: A time-course study in the rat. *Physiology & Behavior*, 92, 734– 740.

Reid, G., Holt, M., Bowman, C., Espelage, D., & Green, J. (2016) Perceived social support and mental health among first-year college students with histories of bullying victimization. *Journal of Child and Family Studies*, 25, 3331–3341.

Rentrop, M., Backenstrass, M., Jaentsch, B., Kaiser, S., Roth, A., Unger, J., Weisbrod, M., & Renneberg, B. (2008) Response inhibition in borderline personality disorder: Performance in a Go/Nogo task. *Psychopathology*, 41, 50-57.

Rescorla, R. (2001) Experimental extinction. In R. Mowrer, & S. Klein (Eds.), *Handbook of Contemporary Learning Theories* (pp. 119-154) Mahwah, NJ: Lawrence Erlbaum Associates.

Retana-Márquez, S., Bonilla-Jaime, H, Vázquez-Palacios, G., Martínez-García, R., & Velázquez-Moctezuma, J. (2003) Changes in masculine sexual behavior, corticosterone and testosterone in response to acute and chronic stress in male rats. *Hormones and Behavior*, 44, 327–337.

Reul, J., & de Kloet, R. (1985) Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology*, 117(6), 2505-2511.

Reul, J., Rothuizen, J., & de Kloet, R. (1991) Age-related changes in the dog hypothalamic-pituitary-adrenocortical system: Neuroendocrine activity and corticosteroid receptors. *The Journal of Steroid Biochemistry and Molecular Biology*, 40(1–3), 63-69.

Rhees, R., Grosser, B., & Stevens, W. (1975) Effect of steroid competition and time on the uptake of (3H) corticosterone in the rat brain: An autoradiographic study. *Brain Research*, 83(2), 293-300.

Robbins, M., & Czekala, N. (1997) A preliminary investigation of urinary testosterone and cortisol levels in wild male mountain gorillas. *American journal of primatology*, 43(1), 51–64.

Robbins, T. W (2002) The 5-choice serial reaction time task: Behavioural pharmacology and functional neurochemistry. *Psychopharmacology*, 163, 362-380.

Rogers, R., Baunez, C., Everitt, B., & Robbins, T. (2001) Lesions of the medial and lateral striatum in the rat produce differential deficits in attentional performance. *Behavioral Neuroscience*, 115(4), 799-811.

Romeo, R., Lee, S., Chhua, N., McPherson, C., & McEwen, B. (2004) Testosterone cannot activate an adult-like stress response in prepubertal male rats. *Neuroendocrinology*, 79,125–132.

Romeo, R., Kaplowitz, E., Ho, A., & Franco, D. (2013) The influence of puberty on stress reactivity and forebrain glucocorticoid receptor levels in inbred and outbred strains of male and female mice. *Psychoneuroendocrinology*, 38, 592-596.

Rossetti, Z., Portas, C., Pani, L., Carboni, S., & Gessa, G. (1990) Stress increases noradrenaline release in the rat frontal cortex: prevention by diazepam. *Eur. J. Pharmac*., 176, 229-231.

Rowell, T. (1961) The family group in golden hamsters: Its formation and break-up. *Behaviour*, 17(2/3), 81-94.

Rubia, K., Russell, T., Overmeyer, S., Brammer, M., Bullmore, E., Sharma, T., Simmons, A., Williams, S., Giampietro, V., Andrew, C., & Taylor, E. (2001) Mapping motor

inhibition: Conjunctive brain activations across different versions of Go/No-Go and stop tasks. *NeuroImage,* 13, 250–261.

Saltzman, W., Schultz-Darken, N., Scheffler, G., Wegner, F., & Abbott, D. (1994) Social and reproductive influences on plasma cortisol in female marmoset monkeys. *Physiology & Behavior,* 56(4), 801-810.

Sangalang, G., & Freeman, H. (976) Steroids in the plasma of the gray seal, *Halichoerus grypus*. *General and Comparative Endocrinology,* 29, 419–422.

Schmidt, M., Enthoven, L., van der Mark, M., Levine, S., de Kloet, E., & Oitzl, M. (2003) The postnatal development of the hypothalamic–pituitary–adrenal axis in the mouse. *International Journal of Developmental Neuroscience,* 21, 125–132.

Schmidt, M., Sterlemann, V., Ganea, K., Liebl, C., Alam, S., Harbich, D., Greetfeld, M., Uhr, M., Holsboer, F., & Müller, M. (2007) Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. *Psychoneuroendocrinology,* 32(5), 417-29.

Schramm, B., Casares, M., & Lance, V. (1999) Steroid levels and reproductive cycle of the Galapagos tortoise, *Geochelone nigra*, living under seminatural conditions on Santa Cruz island (Galapagos). *General and Comparative Endocrinology,* 114, 108–120.

Selden, N., Everitt, B., Jarrari, L., & Robbins, T. (1991) Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience,* 42(2), 335-350.

Seligman, M., & Beagley, G. (1975) Learned helplessness in the rat. *Journal of Comparative and Physiological Psychology*, 88(2), 534-541.

Selye, H (1950) Stress and the general adaptation syndrome. *The British Medical Journal*, 1(4667), 1383-1392.

Seraphin, S., Whitten, P., & Reynolds, V. (2008) The influence of age on fecal steroid hormone levels in male Budongo Forest chimpanzees (*Pan troglodytes schweinfurthii*). *Am. J. Primatol*., 70, 661-669.

Shafiee-Kandjani, A., Arfaei, A., Bozorg-Esfangareh, A., Safikhanlou, S., Arfaei, A., & Jafarzadeh-Gharehziaaddin, M. (2017) Correlates of impulsive and hostile behavior in patients with borderline personality disorder and bipolar II disorder. *J Analytical Research in Clinical Medicine*, 5(1), 26-32.

Sidley, N., & Schoenfeld, W. (1964) Behavior stability and response rate as functions of reinforcement probability on "random ratio" schedules. *Journal of the experimental analysis of behavior*, 7(3), 281-283.

Siegel, H. I. (1985) Aggressive behavior. In H.I Siegel. (Ed) *The hamster* (pp. 261-286) New York: Plenum.

Siegel, H. S. (1980) Physiological stress in birds. *BioScience*, 30(8), 529-534.

Silberg, J., Copeland, W., Linker, J., Moore, A., Roberson-Nay, R., & York, T. (2016) Psychiatric outcomes of bullying victimization: A study of discordant monozygotic twins. *Psychological Medicine*, 46, 1875–1883.

Silk, J. S., Siegle, G. J., Whalen, D. J., Ostapenko, L. J., Ladouceur, C. D., & Dahl, R. E. (2009) Pubertal changes in emotional information processing: Pupillary, behavioral, and subjective evidence during emotional word identification. *Development and psychopathology*, 21(1), 7-26.

Silverman, A., Reinherz, H., & Giaconia, R. (1996) The long-term sequelae of child and adolescent abuse: A longitudinal community study. *Child Abuse & Neglect*, 20(8), 709-723.

Simon, N. W., Beas, B. S., Montgomery, K. S., Haberman, R. P., Bizon, J. L., & Setlow, B. (2013) Prefrontal cortical-striatal dopamine receptor mRNA expression predicts distinct forms of impulsivity. *The European journal of neuroscience*, 37(11), 1779-88.

Skelly, M., Chappell, A., Carter, E., & Weiner, J. (2015) Adolescent social isolation increases anxiety-like behavior and ethanol intake and impairs fear extinction in adulthood: Possible role of disrupted noradrenergic signaling. *Neuropharmacology*., 97, 149-59.

Skodol, A., Siever, L., Livesley, W.J., Gunderson, J., Pfohl, B., & Widiger, T. (2002) The borderline diagnosis II: Biology, genetics, and clinical course. *Biological Psychiatry*, 51, 951–963.

Slawecki, C. (2005) Comparison of anxiety-like behavior in adolescent and adult Sprague–Dawley rats. *Behavioral Neuroscience*, 119(6), 1477–1483.

Smith, L., McDonald, C., Bergstrom, H., Brielmaier, J., Eppolito, A., Wheeler, T., Falco, A., & Smith, R. (2006) Long-term changes in fear conditioning and anxiety-like behavior following nicotine exposure in adult versus adolescent rats. *Pharmacology, Biochemistry and Behavior*, 85, 91–97.

Spackman, D., Riley, V., Monjan, A., & Collector, M. (1978) Corticosterone concentrations in the mouse. *Science, New Series*, 200(4337), 87.

Spiga, F., & Lightman, S. (2015) Dynamics of adrenal glucocorticoid steroidogenesis in health and disease. *Molecular and Cellular Endocrinology*, 408, 227–234.

Spinedi, E., Chisari, A., Pralong, F., & Gaillard, R. (1997) Sexual dimorphism in the mouse hypothalamic-pituitary-adrenal axis function after endotoxin and insulin stresses during development. *Neuroimmunomodulation*, 4(2), 77-83.

St. Aubin, D., Ridgway, S., Wells, R., & Rhinehart, H. (1996) Dolphin thyroid and adrenal hormones: circulating levels in wild and semidomesticated *tursiops truncatus*, and influence of sex, age, and season. *Marine Mammal Science*, 12(1), 1–13.

Stefanski, V. (1998) Social stress in loser rats: Opposite immunological effects in submissive and subdominant sales. *Physiology & Behavior*, 63(4), 605–613.

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., Müller, M., & Schmidt, M. (2008) Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: Implications for stress-related disorders. *Hormones and Behavior*, 53, 386–394.

Strakowski, S., Fleck, D., DelBello, M., Adler, C., Shear, P., Kotwal, R., & Arndt, S. (2010) Impulsivity across the course of bipolar disorder. *Bipolar Disorders*, 12, 285–297.

Stroud, L., Foster, E., Papandonatos, G., Handwerger, K., Granger, D., Kivlighan, K. T., & Niaura, R. (2009) Stress response and the adolescent transition: Performance versus peer rejection stressors. *Development and Psychopathology*, 21(1), 47–68.

Stroud, L., Papandonatos, G., Williamson, D., & Dahl, R. (2011) Sex differences in cortisol response to corticotropin releasing hormone challenge over puberty: Pittsburgh pediatric neurobehavioral studies. *Psychoneuroendocrinology*, 36(8), 1226-1238.

Sturman, D., Mandell, D., & Moghaddam, B. (2010) Adolescents exhibit behavioral differences from adults during instrumental learning and extinction. *Behavioral Neuroscience*, 124(1), 16–25.

Suchecki, D., Rosenfeld, P., & Levine, S. (1993) Maternal regulation of the hypothalamic-pituitary-adrenal axis in the infant rat: The roles of feeding and stroking. *Developmental Brain Research*, 75(2), 185–192.

Sumter, S., Bokhorst, C., Miers, A., Van Pelt, J., & Westenberg, P. (2010) Age and puberty differences in stress responses during a public speaking task: Do adolescents grow more sensitive to social evaluation? *Psychoneuroendocrinology*, 35, 1510—1516.

Takahashi, L., & Lore, R. (1983) Play fighting and the development of agonistic behavior in male and female rats. *Aggressive behavior*, 9, 217-227.

Takahashi, L., Nakashima, B., Hong, H., & Watanabe, K. (2005) The smell of danger: A behavioral and neural analysis of predator odor-induced fear. *Neuroscience and Biobehavioral Reviews*, 29, 1157–1167.

Talpos, J., Wilkinson, L., & Robbins, T. (2006) A comparison of multiple 5-HT receptors in two tasks measuring impulsivity. *Journal of Psychopharmacology*, 20(1), 47 – 58.

Tam, W., Phillips, J., & Lofts, B. (1972) Seasonal changes in the secretory activity of the adrenal gland of the cobra (*Naja naja, Linn*.) *General and Comparative Endocrinology*, 19(2), 218–224.

Tang, M., Lei, J., Sun, X., Liu, G., & Zhao, S. (2013) Stress-induced anhedonia correlates with lower hippocampal serotonin transporter protein expression. *Brain Research*, 1513, 127-134.

Tannock, R., Schachar, R., Carr, R., Chajczyk, D., & Logan, G. (1989) Effects of methylphenidate on inhibitory control in hyperactive children. *Journal of Abnormal Child Psychology*, 17(5), 473-491.

Taravosh-Lahn, K., Bastida, C., & Delville, Y. (2006) Differential responsiveness to fluoxetine during puberty. *Behavioral Neuroscience*, 120(5), 1084 –1092.

Taravosh-Lahn, K., and Delville, Y. (2004) Aggressive behavior in female golden hamsters: Development and the effect of repeated social stress. *Hormones and Behavior*, 46, 428 – 435.

Tidey, J., & Miczek, K. (1996) Social defeat stress selectively alters mesocorticolimbic dopamine release: An in vivo microdialysis study. *Brain Research*, 721(1–2), 140-149.

Tokarz, R., McMann, S., Seitz, L., John-Alder, H., (1998) Plasma corticosterone and testosterone levels during the annual reproductive cycle of male brown anoles (*Anolis sagrei*) *Physiological and Biochemical Zoology*, 71, 139–146.

Toledo-Rodriguez, M., & Sandi, C. (2011) Stress during adolescence increases novelty seeking and risk-taking behavior in male and female rats. *Frontiers in Behavioral Neuroscience*, 5, 17, 1-10.

Toledo-Rodriguez, M., Pitiot, A. Paus, T., & Sandi, C. (2012) Stress during puberty boosts metabolic activation associated with fear-extinction learning in hippocampus, basal amygdala and cingulate cortex. *Neurobiology of Learning and Memory*, 98, 93–101.

Tripp, K., Verstegen, J., Deutsch, C., Bonde, R., de Wit, M., Manire, C., Gaspard, J., & Harr, K. (2010) Evaluation of adrenocortical function in florida manatees (*Trichechus manatus latirostris*) *Zoo Biology*, 29, 1–15.

Truscott, B., & Idler, D. (1972) Corticosteroids in plasma of elasmobranchs. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 42, 41-50.

Tsoory M., & Richter-Levin G. (2005) Learning under stress in the adult rat is differentially affected by "juvenile" or "adolescent" stress. *International Journal of Neuropsychopharmacology*, 1–16.

Tyrrell, C., & Cree, A. (1994) Plasma corticosterone concentrations in wild and captive juvenile tuatara (*Sphenodon punctatus*). *New Zealand Journal of Zoology*, 21(4), 407-416,

Tzanoulinou, S., Riccio, O., de Boer, M.W., & Sandi, C. (2014) Peripubertal stress-induced behavioral changes are associated with altered expression of genes involved in excitation and inhibition in the amygdala. *Translational Psychiatry*, 4.

Valente, A., Velarde, R., Parga, M., Marco, I., Lavin, S., Alegre, F., & Cuenca, R. (2011) Reproductive status of captive Loggerhead sea turtles based on serum levels of gonadal steroid hormones, corticosterone and thyroxin. *Veterinary Journal*, 187(2), 255-259.

van der Bergh, F., Bloemarts, E., Groenink, L., Olivier, B., & Oosting, R. (2006) Delay aversion: Effects of 7-OH-DPAT, 5-HT1A/1B receptor stimulation and D-cycloserine. *Pharmacology Biochemistry & Behavior*, 85(4), 736–743.

van Gaalen, M., Brueggeman, R., Bronius, P., Schoffelmeer, A., & Vanderschuren, L. (2006) Behavioral disinhibition requires dopamine receptor activation. *Psychopharmacology*, 187, 73-85.

van Gaalen, M., van Koten, R., Schoffelmeer, A., & Vanderschuren, L. (2006) Critical involvement of dopaminergic neurotransmission in impulsive decision making. *Biological Psychiatry*, 60(1), 66-73.

van Kampen, M., & Fuchs, E. (1998) Age-related levels of urinary free cortisol in the tree shrew. *Neurobiology of Aging*, 19(4), 363-366.

Varlinskaya, E.I., Spear, L.P., & Spear, N.E. (1999) Social Behavior and Social Motivation in Adolescent Rats: Role of Housing Conditions and Partner's Activity. *Physiol Behav*, 67, 475-482.

Vazquez, D., & Akil, I. (1993) Pituitary-adrenal response to ether vapor in the weanling animal: Characterization of the inhibitory effect of glucocorticoids on adrenocorticotropin secretion. *Pediatric research*, 34(5), 646-653.

Veenema, A. (2009) Early life stress, the development of aggression and neuroendocrine and neurobiological correlates: What can we learn from animal models? *Frontiers in Neuroendocrinology*, 30, 497–518.

Velley, L., & Cardo, B. (1982) Facilitation of acquisition and extinction of an operant task four weeks after stimulation of brainstem aminergic nuclei of the rat. *Behavioral and Neural Biology*, 35, 395-407.

Vidal, J., Buwalda, B., & Koolhaas, J. (2011a) Differential long-term effects of social stress during adolescence on anxiety in Wistar and Wild-type rats. *Behavioural Processes*, 87, 176–182.

Vidal, J., Buwalda, B., & Koolhaas, J. (2011b) Male Wistar rats are more susceptible to lasting social anxiety than Wild-type Groningen rats following social defeat stress during adolescence. *Behavioural Processes*, 88(2), 76-80.

Vidal, J., de Bie, J., Granneman, R., Wallinga, A., Koolhaas, J., & Buwalda, B. (2007) Social stress during adolescence in Wistar rats induces social anxiety in adulthood without affecting brain monoaminergic content and activity. *Physiology & Behavior*, 92, 824–830.

Vomachka, A., & Greenwald, G. (1979) The development of gonadotropin and steroid hormone patterns in male and female hamsters from birth to puberty. *Endocrinology*, 105, 960–966.

Wada, H., Hahn,T., & Breuner, C. (2007) Development of stress reactivity in white-crowned sparrow nestlings: Total corticosterone response increases with age, while free corticosterone response remains low. *General and Comparative Endocrinology*, 150(3), 405-413.

Wahlstrom, D., White, T., & Luciana, M. (2010) Neurobehavioral evidence for changes in dopamine system activity during adolescence. *Neuroscience & Biobehavioral Reviews*, 34(5), 631-648.

Walker, C., Perrin, M., Vale, W., & Rivier, C. (1986) Ontogeny of the stress response in the rat: Role of the pituitary and the hypothalamus. *Endocrinology*, 118(4), 1445-1451.

Walker, E., Sabuwalla, Z., & Huot, R. (2004) Pubertal neuromaturation, stress sensitivity, and psychopathology. *Development and Psychopathology*, 16, 807–824.

Wallin, K., Alves, J., & Wood, R. (2015) Anabolic-androgenic steroids and decision making: Probability and effort discounting in male rats. *Psychoneuroendocrinology*, 57, 84–92.

Wallin-Miller, K., Li, G., Kelishani, D., & Wood, R.I. (2018) Anabolic-androgenic steroids after decision making in a balanced rodent model of the Iowa gambling task. *Behav Neurosci*, 132(3), 152-160.

Watt, M., Burke, A., Renner, K., & Forster, G. (2009) Adolescent male rats exposed to social defeat exhibit altered anxiety behavior and limbic monoamines as adults. *Behavioral Neuroscience*, 123 (3), 564 –576

Watt, M., Roberts, C., Scholl, J., Meyer, D., Miiller, L., Barr, J., Novick, A., Renner, K., & Forster, G. (2014) Decreased prefrontal cortex dopamine activity following adolescent social defeat in male rats: Role of dopamine D2 receptors. *Psychopharmacology*, 231, 1627–1636.

Watt, M., Weber, M., Davies, S., & Forster, G. (2017) Impact of juvenile chronic stress on adult cortico-accumbal function: Implications for cognition and addiction,

Progress in Neuro-Psychopharmacology and Biological Psychiatry, 79, Part B, 136-154,

Whiteside, S., Lynam, D., Miller, J., & Reynolds, S. (2005) Validation of the UPPS impulsive behaviour scale: A four-factor model of impulsivity. *Eur. J. Pers.*, 19, 559–574.

Widmaier, E., & Kinz, T. (1993) Basal, diurnal, and stress-induced levels of glucose and glucocortiocids in captive bats. *Ecological and integrative physiology*, 265(5), 533-540.

Wildt, D., Phillips, L., Simmons, L., Chakraborty, P., Brown, J., Howard, J., Teare, A., & Bush, M. (1988) A comparative analysis of ejaculate and hormonal characteristics of the captive male cheetah, tiger, leopard, and puma. *Biology of Reproduction*, 38(2), 245–255.

Willner, P., Towell, A., Sampson, D, Sophokleous, S., & Muscat, R. (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology*, 93 (3), 358–364.

Winstanley, C., Chudasama, Y., Dalley, J., Theobald, D., Glennon, J., & Robbins, T. (2003) Intra-prefrontal 8-OH-DPAT and M100907 improve visuospatial attention and decrease impulsivity on the five-choice serial reaction time task in rats. *Psychopharmacology*, 167, 304–314.

Winstanley, C., Dalley J., Theobald, D., & Robbins T. (2003) Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. *Psychopharmacology*, 170, 320–331.

Winstanley, C., Dalley J., Theobald, D., & Robbins T. (2004) Fractionating impulsivity: Contrasting effects of central 5-HT depletion on different measures of impulsive behavior. *Neuropsychopharmacology*, 29(7), 1331-1343.

Winstanley, C., Theobald, D., Dalley, J., & Robbins, T. (2005) Interactions between serotonin and dopamine in the control of impulsive choice in rats: Therapeutic implications for impulse control disorders. *Neuropsychopharmacology*, 30, 669–682.

Winstanley, C., Theobald, D., Dalley, J., Glennon, J., & Robbins, T. (2004) 5-HT2A and 5-HT2C receptor antagonists have opposing effects on a measure of impulsivity: Interactions with global 5-HT depletion. *Psychopharmacology*, 176(3–4), 376–385.

Wogar, M., Bradshaw, C., & Szabadi, E. (1993) Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on choice between delayed reinforcers. *Psychopharmacology*, 11, 239-243.

Wommack, J., & Delville, Y. (2003) Repeated social stress and the development of agonistic behavior: Individual differences in coping responses in male golden hamsters. *Physiology & Behavior*, 80(2–3), 303–308.

Wommack, J., & Delville, Y. (2007) Cortisol controls the pubertal development of agonistic behavior in male golden hamsters via type II corticosteroid receptors. *Hormones and Behavior*, 51(3), 306-312.

Wommack, J., Salinas, A., & Delville, Y. (2005) Glucocorticoids and the development of agonistic behaviour during puberty in male golden hamsters. *Journal of Neuroendocrinology*, 17, 781–787.

Wommack, J., Salinas, A., Melloni., R., & Delville, Y. (2004) Behavioural and neuroendocrine adaptations to repeated stress during puberty in male golden hamsters. *Journal of Neuroendocrinology*, 16, 767–775.

Wommack, J., Taravosh-Lahn, K., David, J., & Delville, Y. (2003) Repeated exposure to social stress alters the development of agonistic behavior in male golden hamsters, *Hormones and Behavior*, 43, 229–236.

Wood, R., Armstrong, A., Fridkin, V., Shah, V., Najafi, A., & Jakowec, M. (2013) 'Roid rage in rats? Testosterone effects on aggressive motivation, impulsivity and tyrosine hydroxylase. *Physiology & Behavior*, 0, 6–12.

Wright, R., & Conrad, C. (2005) Chronic stress leaves novelty-seeking behavior intact while impairing spatial recognition memory in the Y-maze. *Stress*, 8(2), 151–154.

Yap, J. J., Chartoff, E. H., Holly, E. N., Potter, D. N., Carlezon, W. A., & Miczek, K. A. (2014) Social defeat stress-induced sensitization and escalated cocaine self-administration: The role of ERK signaling in the rat ventral tegmental area. *Psychopharmacology*, 232(9), 1555-69.

Yates, J., Perry, J., Meyer, A., Gipson, C., Charnigo, R., & Bardo, M. (2014) Role of medial prefrontal and orbitofrontal monoamine transporters and receptors in performance in an adjusting delay discounting procedure. *Brain Research*, 1574, 26–36.

Zeeb, F., Floresco, S., & Winstanley, C. (2010) Contributions of the orbitofrontal cortex to impulsive choice: Interactions with basal levels of impulsivity, dopamine signalling, and reward-related cues. *Psychopharmacology*, 211(1), 87–98.

Zerani M1, Gobbetti A. (1993) Corticosterone during the annual reproductive cycle and in sexual behavior in the crested newt, *Triturus carnifex*. *Hormones and Behavior*, 27(1), 29-37.

Zhang, F., Yuan, S., Shao, F., & Wang, W. (2016) Adolescent social defeat induced alterations in social behavior and cognitive flexibility in adult mice: Effects of developmental stage and social condition. *Frontiers in Behavioral Neuroscience*, 10, 149.

Zipser, B., Schleking, A., Kaiser, S., & Sachser, N. (2014) Effects of domestication on biobehavioural profiles: a comparison of domestic guinea pigs and wild cavies from early to late adolescence. *Frontiers in zoology*, 11(1), 30.

Zolovick, A., Upson, D., & Eleftheriou, B. (1966) Diurnal variation in plasma glucocorticosteroid levels in the horse (*Equus Caballus*) *Journal of Endocrinology*, 35, 249-253.

Zou, S., Funk, D., Shram, M., & Lê, A. (2014) Effects of stressors on the reinforcing efficacy of nicotine in adolescent and adult rats. *Psychopharmacology*, 231(8), 1601–1614.